REVIEW ARTICLE

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New Approaches to the Treatment of Inflammatory Disease

Focus on Small-Molecule Inhibitors of Signal Transduction Pathways

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Contents

Abstract 397
1. Small-Molecule Inhibitors of Alternative Pro-Inflammatory Signalling Cascades 399
   1.1 Nuclear Transcription Factor (NF-κB) Signalling Pathway 400
      1.1.1 NF-κB Inhibitors 402
   1.2 P38 Mitogen-Activated Protein Kinase (MAPK) Signalling Pathway 414
      1.2.1 P38 MAPK Inhibitors 416
   1.3 Janus Protein Tyrosine Kinase (JAK)/Signal Transducers and Activators of Transcription (STAT) Signalling Pathway 423
      1.3.1 JAK/STAT Inhibitors 424
2. Conclusion 427

Abstract

This 'state-of-the-art' review specifically focuses on alternative signalling pathways deeply involved in acute and chronic inflammatory responses initiated by various pathological stimuli. The accumulated scientific knowledge has already revealed key biological targets, such as COX-2, and related pro-inflammatory mediators (cytokines and chemokines, interleukins [ILs], tumour necrosis factor [TNF]-α, migration inhibition factor [MIF], interferon [IFN]-γ and matrix metalloproteinases [MMPs]) implicated in uncontrolled, destructive inflammatory reaction. A number of physiologically active agents are currently approved for market or are under active investigation in different clinical trials. However, recent findings have exposed the fatal adverse effects directly associated with drug therapy based on COX-2 inhibition. Given these possible harmful outcomes, a range of novel therapeutically relevant biological targets that include nuclear transcription factor (NF-κB), p38 mitogen-activated protein kinases (MAPK) and Janus protein tyrosine kinases and signal transducers and activators of transcription (JAK/STAT) signalling pathways has received growing attention. Here we discuss recent progress in the identification and development of novel, clinically approved or evaluated small-molecule regulators of these signalling cascades as promising anti-inflammatory drugs.

In the original conception, inflammation (Latin, *inflammatio*, to set on fire) is an extremely complex biological response of the human organism to a variety of harmful stimuli, such as pathogens, foreign substances (bacteria and viruses), damaged cells and wounds, infection, viruses and irritants; the body sends out signals that normal tissues are infected, damaged or somehow abnormal. From the functional point of view the organism painstakingly attempts to remove injurious stimuli as well as initiate the self-healing protective defence against infection. However, in some diseases, the immune system inappropriately triggers an inflammatory response when no foreign substances or infection are present. In these autoimmune conditions the body's defence mechanism causes huge damage to its own tissues, sustaining the perpetual or transitory activation of the inflammatory process. Abnormalities associated with uncontrolled inflammation comprise a large, unrelated group of disorders that underlie a variety of dangerous human diseases, such as rheumatoid arthritis (RA), ischaemic heart disease, atherosclerosis, cancer, fibrosis and hay fever, as well as several neurodegenerative disorders including Parkinson's and Alzheimer's diseases. To avoid these harmful outcomes, inflammation is normally tightly controlled by a variety of regulating factors.

The pathophysiology and treatment of diseases accompanied or initiated/sustained by harmful inflammation have been revolutionized by the discovery of common pro-inflammatory effector mechanisms involving a large variety of signalling molecules, specific proteins and transcription factors, most of which are susceptible to a genetic mutation that significantly impairs or otherwise dysregulates normal cellular function and gene expression. Among a huge number of pro-inflammatory mediators, cyclo-oxygenase 1 and 2 (COX-1/2), mitogen-activated protein kinases (MAPKs), Janus protein tyrosine kinases (JAKs), nuclear transcription factor (NF-κB) and signal transducers and activators of transcription (STAT) are the principal effectors that directly or indirectly lead to the production of a vast number of pro-inflammatory cytokines and regulatory proteins such as interleukin (IL)-1/6, tumour necrosis factor (TNF)-α, migration inhibition factor (MIF), interferon (IFN)-γ, and matrix metalloproteinases (MMPs).[1] These messengers, in turn, jointly support inflammatory processes, contributing to both homeostatic and pathological outcomes.

In the early 1990s anticytokine therapies were started and fully confirmed the critical role of these molecules in autoimmune diseases. During the last decade, our knowledge of the molecular basis of inflammation has been considerably broadened and much is actually known about the key role of pro-inflammatory mediators.[2,3] The accumulated findings are currently opening exciting doors to new horizons for the drug treatment of inflammatory-associated diseases through selective targeting of specific pro-inflammatory factors. Thus, an existing theoretical and practical experience has already helped thousands of patients who were treated with novel anti-inflammatory drugs, years before they became routinely available; hundreds of promising anti-inflammatory agents are currently being evaluated in different clinical trials (based on a thorough analysis of the Prous Ensemble Database; internet site: URL: http://www.prous.com).

Historically, anti-inflammatory drugs had their origins in the serendipitous discovery of certain plants and application of their extracts to relieve pain, fever and inflammation. When salicylates were discovered in the mid-19th century, this enabled these compounds to be synthesized and later modified as acetylsalicylic acid (aspirin). This was followed by a period marked by the 'triumph' of corticosteroid-based anti-inflammatory compounds. Likewise, the chemical advances of the 19–20th centuries led to the development of the nonsteroidal anti-inflammatory drugs (NSAIDs) or nonsteroidal anti-inflammatory medicines, most of which were initially organic acids, although non-acidic compounds were later also discovered. The most prominent members of this group of drugs are aspirin, ibuprofen and naproxen, partly because they are available 'over the counter' in many areas. Part of the popularity of NSAIDs is that, unlike opioids, they do not produce sedation or respiratory depression and have a very low addiction rate. However,

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many clinically approved drug compounds have recently been found to have significant adverse effects leading to increased clinical risk and various dangerous complications, including bleeding, ulceration and perforation of the stomach or intestines, which can be fatal. Indeed, a number of developed anti-inflammatory agents showed a high incidence of gastrointestinal (the principal adverse reactions directly associated with NSAID usage) and renal adverse effects and atherothrombosis.[4] These effects are dose dependent, and in many cases severe enough to pose the risk of ulcer perforation, upper gastrointestinal bleeding and death, significantly limiting the clinical application of many NSAIDs. It has been estimated that up to 25% of NSAID patients experience dyspepsia,[5] and NSAID-associated upper gastrointestinal adverse events result in hundreds of thousands of hospitalizations and more than 10 000 deaths per year around the world.[6]

As a result of research focused on reduction of the adverse effects of NSAIDs, selective COX-2 inhibitors, such as celecoxib and rofecoxib, were recently developed. However, efforts are currently underway not only to partly or completely overcome the fatal cardiovascular reactions associated with use of these agents, but also to elucidate the roles of COX-2 and COX-1 in cardiovascular disease and stroke in the hope that there may be some basis for developing novel agents (e.g. nitric oxide-donating NSAIDs) to appropriately control these conditions. Many data clearly demonstrate that the mechanisms of action of selective COX-2 inhibitors are multidirectional and, in reality, rather complex. Lack of responsiveness, various adverse effects as well as resistance to many of the currently existing anti-inflammatory drugs mean that the design and development of novel anti-inflammatory agents is still absolutely necessary.

In response to all the potential risks listed, an advanced generation of novel anti-inflammatory drugs acting primarily against pro-inflammatory mediator production is being discovered and developed based on the effects of these agents on alternative signal transduction pathways. These drugs are currently being heralded as ‘new-age’ therapies to control those diseases in which these alternative factors and signalling molecules as well as other nonprostaglandin components of chronic inflammatory and neurodegenerative diseases are becoming manifest. What started out as drugs to control inflammation, pain and fever in the last two centuries has now exploded to reveal an enormous range and type of anti-inflammatory agents and discovery of new prominent therapeutic targets to treat a whole range of conditions that were never hitherto envisaged. Among these biological targets, NF-κB, p38 MAPK and JAK/STAT signalling molecules represent the most promising avenue for achieving optimal therapeutic response with minimal adverse effects.

1. Small-Molecule Inhibitors of Alternative Pro-Inflammatory Signalling Cascades

The pro-inflammatory signalling pathways and cellular mechanisms that initiate the normal inflammatory response have become increasingly well characterized. However, the therapeutic arms by which uncontrolled and destructive inflammation can be temporarily or permanently switched off remain unclear because of the hugely complex and intricate interrelationships among a multitude of pro-inflammatory mediators. Recent data clearly indicate that resolution of inflammation is an active process controlled by a vast number of endogenous factors that can either activate or suppress pro-inflammatory gene expression and cell trafficking as well as strongly regulate inflammatory-cell apoptosis and phagocytosis. Recent progress that has been made in our understanding of inflammatory signalling cascades has already identified a series of novel promising targets, notably in pathways involving NF-κB and JAK/STAT transcription factors, p38 MAPK, glycogen synthase kinase-3 (GSK3) and IκB kinase (IKK) activation, phospholipase A2 (PLA2), caspases and various growth factors and their receptors (GF-Rs), high-mobility group box 1 (HMGB1), phosphodiesterase 4 (PDE4), mast cells and related transient receptor potential vanilloid (TRPV) channels, T-lymphocyte and platelet activa-

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tion, MMPs, corticosteroids, T-cell immunoglobulin mucin 3 (TIM-3), IL-17/18/27, TWEAK, toll (TLR) and toll-like (TLR-like) receptors as well as Wnt/Frizzled signalling route. For example, it was clearly demonstrated that in addition to their proteolytic activity, caspases can participate via their caspase recruitment domain (CARD) in signalling complexes leading to NF-κB and p38 MAPK activation.[7] Other biological targets such as the key components and messenger molecules implicated in pathways activated via specific transmembrane TNF receptor (TNFR), G-protein coupled receptor (GPCR), B-cell receptor (BCR), lymphotoxin β receptor (LTβR) and Nod-like receptors (NLRs) also represent promising therapeutic targets for management of inflammation, and might show efficacy without being limited by effects on host defence. The prime challenge is to better understand the principal role of each target, and to devise effective strategies to modulate their activities through small-molecule agents or naturally derived compounds.

For this study, we undertook a thorough literature search and evaluation across a variety of available on-line pharmaceutical sources (free-opened and commercial), including Prous Ensemble, WOMBAT (World of Bimolecular Activity),[8] Beilstein[9] and National Center for Biotechnology Information (URL: http://www.pubmed.com) databases, as well as the internet sites of specialized journals cited in the current review.

1.1 Nuclear Transcription Factor (NF-κB) Signalling Pathway

The NF-κB intracellular signalling system seems to be becoming the dominant paradigm for specific signal transduction molecules, regulatory proteins and gene activation in response to inflammatory and menacing stimuli. Especially during initial hyper-inflammatory states of an acute illness, such as sepsis, or in the course of chronic inflammation and autoimmune diseases, inhibition of IKK-driven NF-κB activation provides a promising therapeutic strategy. The spectrum of NF-κB target genes include primarily those that are responsible for mediators and effectors of both innate and adaptive immunity and inhibitors of apoptosis, growth-promoting factors and virus-encoded proteins involved in viral replication, as well as self-regulatory proteins for NF-κB actions.[10] In addition to its role in the original inflammatory conditions, NF-κB signalling pathway is heavily involved in the onset of various inflammatory-related autoimmune disorders and different types of cancer.[11] Indeed, it has recently been reported[12] that NF-κB plays major roles in leukaemia, inflammatory bowel disease, arthritis, sepsis, asthma, multiple sclerosis, colitis, diabetic neuropathy[13] and AIDS. For example, RA pathology was found to be mediated by a number of cytokines (TNFα, IL-1/6/17, IFNγ, etc.), chemokines (monocyte chemoattractant protein [MCP]-1/4, C-C motif ligand [CCL]-18, etc.), cell adhesion molecules (intercellular cell adhesion molecule [ICAM]-1, vascular cell adhesion molecule [VCAM]-1, etc.) and MMPs. Thus, in patients diagnosed with RA, activation of NF-κB signalling pathway results in the transcription of a multitude of responsive genes that contribute to the inflammatory phenotype, including TNFα, IL-6 and MMPs that, in turn, recruit immune cells to the inflamed pannus. This is largely a consequence of activation of the canonical NF-κB pathway that leads finally to the formation of heterodimeric transcriptional units composed of different p/p complexes that directly initiate gene expression.[14]

Notwithstanding the fact that there are significant differences in the signalling networks of human and murine immune cells and immortalized cell lines, much information on the role of NF-κB in inflammation has been gleaned from deficiencies of the respective genes in mice. Despite these differences at the molecular level, major interest has been focused on recent animal studies and data derived from genetic analysis in humans that overwhelmingly indicate that signalling via NF-κB is a key process controlling inflammation and thereby constitutes an attractive target for anti-inflammatory therapeutic interventions.[15] Great commercial and scientific efforts have already gone into developing highly specific and multitargeted inhibitors of NF-κB activation, some of which are currently being evaluated

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in different clinical trials. However, inhibition of the NF-κB signalling route can result in an exacerbation of inflammation if TNFα production by macrophages is not completely controlled. In addition, there is a certain amount of risk in the form of induced immunodeficiency that may follow inhibitory treatment. Moreover, its primary anti-apoptotic function suggests that blockage of NF-κB activation has dramatic effects on cell functions and survival, and eventually worsens the course of an inflammatory disease. Thus, it will be very important to carefully monitor use of such inhibitors before they are used as long-term treatments in patients with chronic inflammatory conditions. The relative pros and cons of NF-κB inhibition and corresponding drug therapy have already been critically reviewed.[16]

Because of space constraints we have only elucidated the key aspects of the NF-κB signalling system in this review. A schematic diagram of the canonical and alternative NF-κB pathways is shown in figure 1. While the canonical route is directly activated by many well known receptor systems such as GPCR and TNFR, signalling through a subset of unique receptors, including LTβR, CD40 and BlyS receptor (BR)-3, activates the alternative NF-κB signalling pathway via the activation of NF-κB-inducing kinase (NIK), which in turn directly stimulates IKK complexes (see below). Recent enzymatic and genetic studies have shown that the NF-κB family is composed of five principal members, including NF-κB1/(p50), NF-κB2/(p52), RelA/(p65), RelB and c-Rel/(Rel) [15 possible dimers]. Hetero- and homo-dimerization observed among these nuclear factors that exhibit differential DNA-binding specificities and different transactivation potential led to the formation of the following transcriptional complexes: p50/RelA, p50/c-Rel, p52/c-Rel, p65/c-Rel, RelA/RelA, p50/p50, p52/p52, RelB/p50 and RelB/p52. Activation of NF-κB is thought to be part of a stress response because it occurs in response to a variety of stimuli that include inflammatory cytokines such as TNFα and IL-1/6, growth factors, lipopolysaccharides (LPS, the main components of bacterial cell walls) or lymphokines, oxidant-free radicals, inhaled particles, viral infection or expression of certain viral or bacterial gene products, UV irradiation, B- or T-cell activation, pharmacological agents, different stress conditions as well as other physiological and non-physiological stimuli.[17] Depending on the stimulus, the mechanism of activation involves several overlapping and non-overlapping steps, as briefly overviewed in figure 1. Among the various stimuli known to date, IL-1- and TNF-induced activation of NF-κB have been studied in greater detail. In general, the TNF/NF-κB pathway involves the interaction of the ligand with TNFR, which then recruits the TNFR-associated death domain (TRADD) protein. This protein binds to TNFR-associated factor-2 (TRAF2), which, in turn, activates receptor-interacting protein (RIP). RIP recruits and directly activates NIK and MAPK kinases (MEKKs), thereby inducing rapid phosphorylation of IKK. NF-κB dimers are sequestered in the cytosol of unstimulated cells via non-covalent interactions with a class of inhibitor molecules, called IκBs, which form the protein conglomerate IKK. IKK, in turn, includes three subunits: IKK-α (IKK-1, a predominant IκB kinase), IKK-β (IKK-2) and IKK-γ (also known as NEMO) that has no catalytic activity but plays a critical regulatory role. To date seven IκBs have been identified: IκB-α, IκB-β, IκB-γ, IκB-ε, B cell leukaemia/lymphoma (Bcl)-3, p100 and p105. These complexes prevent nuclear translocation of NF-κB into the nucleus until signals that induce NF-κB activity cause the phosphorylation of IκBs, their dissociation and subsequent degradation, thereby releasing the NF-κB active complex.

Degradation of IκB proteins is also carried out by the proteasome but only after prior phosphorylation of IκB by the IKK. The phosphorylated IκB is further specifically recognized by β-transducin repeat containing protein (β-TrCP), a component of the ubiquitin ligase complex, and TRAF6, which jointly regulate poly-ubiquitination of IκB and its subsequent proteasomal degradation. In the long run, the 'shackle-free' NF-κB/Rel complexes translocate into the cell nucleus where, either alone or in combination with other transcription factor families

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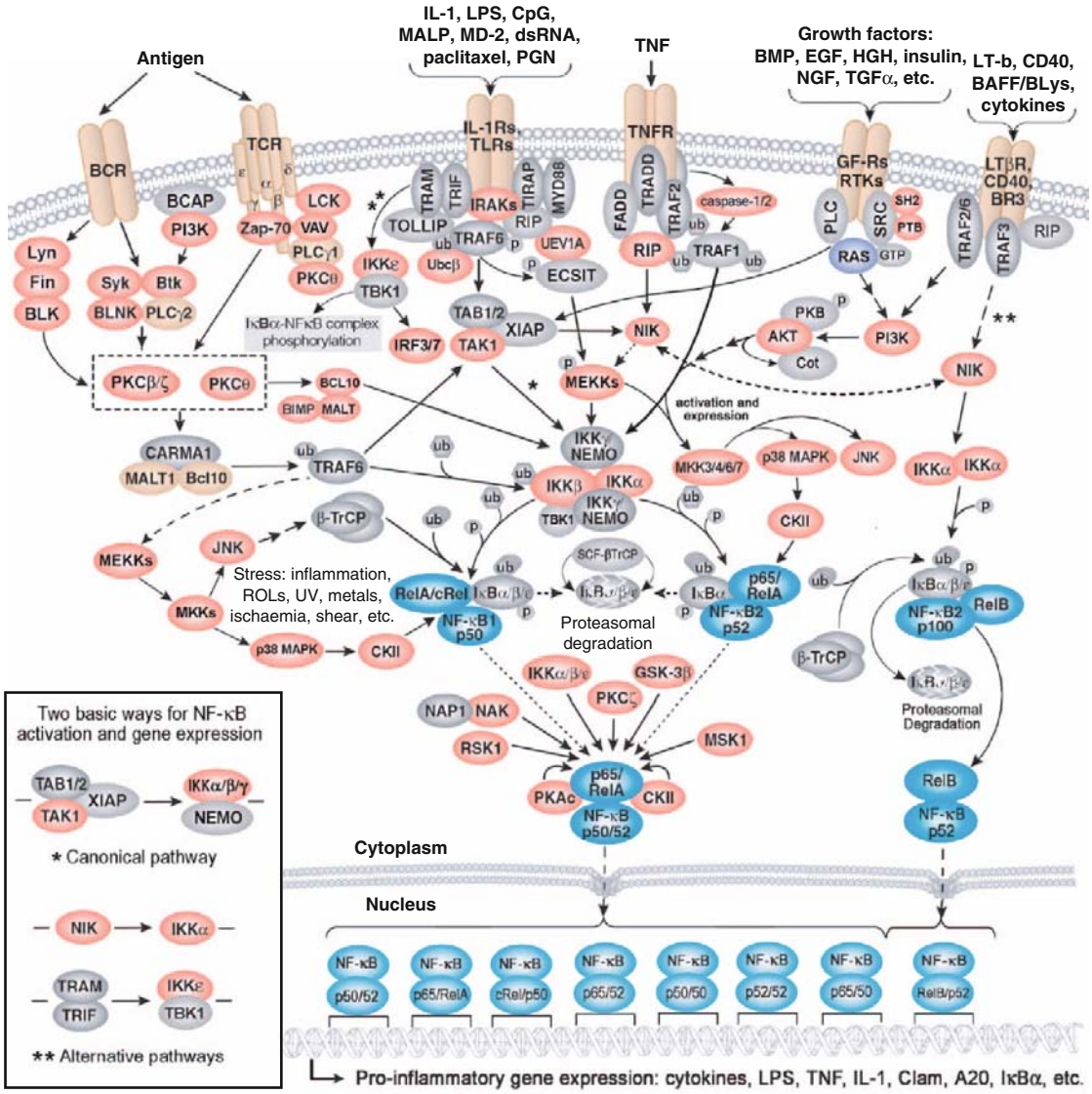

Fig. 1. Canonical and alternative nuclear transcription factor (NF-κB) signalling pathways implicated in inflammation. Please refer to text for definition of relevant abbreviations.

including activator protein-1(AP-1), Ets and STAT, induce pro-inflammatory gene expression such as cytokines IL-1/6, TNFα and LPS, which, in turn, restimulate and maintain inflammation.

1.1.1 NF-κB Inhibitors

In past decades, enormous resources have been recruited to invent, develop and apply novel therapeutics against inflammation. Among numerous compounds that were found to have considerable physiological and therapeutic significance against different inflammation conditions acting directly on the NF-κB protein complexes or NF-κB-related signalling pathways, plant-derived agents (extracts and essence), corticosteroid-based compounds and several small-molecule mediators jointly compose a

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large therapeutic group. Recent advances achieved in different preclinical models have clearly identified a wide therapeutic potential of many small-molecule NF-κB inhibitors, neutralizing antibodies/proteins or genetically altered gene functions against various inflammatory mediators. Several clinically approved drug compounds have been launched onto the world pharmaceutical market. Table I summarizes the main characteristics of clinically proven small-molecule NF-κB inhibitors developed by various pharmaceutical firms. Several inhibitors have been found to have improved therapeutic potential when used in combination.

Oxycodone/Ibuprofen (Combunox®)

Combunox®, a combination of oxycodone (a semisynthetic opioid analgesic) and ibuprofen (an NSAID with analgesic and antipyretic properties), was initially launched by Forest in 2005 for the short-term (up to 7 days) treatment of acute, moderate to severe pain.[18] Originally developed by BTG (London, UK), the product was exclusively licensed to Forest (New York, NY, USA) for manufacturing, sales and marketing in the US. Currently, Combunox® is positioned as an oral fixed-dose combination tablet with analgesic, anti-inflammatory and antipyretic properties. The key anti-inflammatory component is ibuprofen, the mode of action of which is still not completely understood but, presumably, is similar to that of other NSAIDs, and is thought to be related to its inhibition of COX activity and prostaglandin (PG) synthesis. In addition, several recent studies strongly suggest that the prime activity of ibuprofen is directly associated with its ability to inhibit NF-κB activation (see below).[19] Importantly, Combunox® is generally well tolerated after single or multiple doses and short-term usage is not expected to produce any of the serious adverse effects typically associated with long-term treatment with opioids or NSAIDs. Therefore, Combunox® is an effective, convenient treatment option for the short-term management of acute, moderate to severe pain and different inflammatory conditions.

Bortezomib

Bortezomib is a potent proteasome inhibitor originally launched in the US in 2003 by Millennium (Cambridge, MA, USA) for the treatment of multiple myeloma. Since then, the product has been approved and commercialized in several countries worldwide. As described above, the ubiquitin-proteasome system (UPS) has a central role in the selective degradation of intracellular proteins including those deeply implicated in inflammatory processes, such as IκB. The UPS also regulates inflammatory responses via the upregulation of several pro-inflammatory molecules. Consequently, proteasome inhibition is a potential therapeutic strategy against cancer and coupled inflammatory conditions. Indeed, bortezomib has been found to effectively block multi-ubiquitinated protein degradation by inhibiting 26S proteasome activity involved in cell-cycle regulation, cellular apoptosis, inflammation and immune surveillance.[20] Bortezomib-induced proteasomal inhibition leads to prolonged storage of NF-κB/IκB complexes, resulting in significant decrements in mRNA expression of TNFα and IL-6 and decreased production of inducible nitric oxide synthase (iNOS) and COX-2.[21] In multiple myeloma cells, bortezomib directly induces cell stress response followed by activation of c-Jun NH(2) terminal kinase (JNK)/stress-activated protein kinase (SAPK), and triggers caspase-dependent apoptosis of tumour cells.[22] In addition, bortezomib, as well as several other NF-κB inhibitors including dexamethasone and thalidomide, also showed inhibitory effects on LPS-induced growth arrest and DNA damage-inducible family of genes (GADD45β) expression involved in the regulation of cell-cycle progression and apoptosis as well as acute inflammation.[23]

Pranlukast

Pranlukast was originally launched in Japan in 1995 as a potential small-molecule antagonist of cysteinyl leukotriene receptor 1 (CysLT1) and CysLT2 activities deeply implicated in many inflammatory disorders such as chronic airway inflammation, bronchial asthma and allergic rhinitis.[24] The effects of the leukotrienes are mediated through receptors that are expressed on a variety of cell types and can be modulated based on the inflammatory environment present. In addition, it

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Table I. The main characteristics of clinically proven small-molecule nuclear transcription factor (NF-κB) inhibitors

Compound	Development phase	Therapeutic targets	Mechanism of action	Originator(s)
Combunox® (oxycodone/ibuprofen)[a]	Launched 2005	Nonspecific inflammation	Multitargeted inhibitor, especially against NF-κB and COX-1/2/3	BTG
Bortezomib (MLN-341)	Launched 2003	Nonspecific inflammation and cancer	NF-κB, AP-1 and proteasome inhibitor	Millennium Pharmaceuticals and Janssen-Cilag
Pranlukast (RS-411)	Launched 1995	Upper respiratory tract disorders, COPD, asthma, allergic rhinitis	Multitargeted inhibitor, especially against NF-κB and leukotriene CysLT2/ CysLT1	Ono
Fumaderm® (dimethyl fumarate/(Ca, Mg, Zn) monoethyl fumarates)[a]	Launched 1994	Psoriasis and multiple sclerosis	NF-κB targeted inhibitor	Biogen Idec
Gabexate	Launched 1978	Pancreatic disorders	Multitargeted inhibitor, especially against NF-κB and AP-1	Ono
Sulindac (MK-231)	Launched 1976	Ankylosing spondylitis, rheumatoid arthritis, gout, osteoarthritis	NF-κB inhibitor and ABCC1/3 expression enhancer	Merck
Silibinin (one of the major active constituents of silymarin)	Launched 1972	Liver and biliary tract disorders, lipoprotein disorders, disorders of the coronary arteries and atherosclerosis, diabetes mellitus, viral hepatitis	Multitargeted inhibitor especially against NF-κB, HMG-CoA reductase, reverse transcriptase as well as ApoB secretion	Madaus
Ibuprofen (ST-1482, ZAG-1701)	Launched 1969	Ankylosing spondylitis, rheumatoid arthritis, osteoarthritis	NF-κB and COX-1/2/3 inhibitor	Zambon and Merckle GmbH
Acetylcysteine (RK-0202, NSC-111180)	Launched 1968	Renal failure, interstitial lung diseases, inflammatory bowel disease, OCD, metabolic disorders (not specified), psychiatric disorders (not specified), cardiovascular diseases, COPD, cocaine dependency, preterm labour, mucositis	NF-κB targeted inhibitor	Zambon and Yale University
Sulfasalazine (salazosulfapyridine)	Launched 1944	Inflammatory bowel disease and rheumatoid arthritis	NF-κB targeted inhibitor	Pfizer
Mepacrine (CBLC-102)	Launched 1932	Prostate and renal cancer therapy, malaria, protozoal diseases, prion diseases	Multitargeted inhibitor, particularly against NF-κB and secretory PLA2	Bayer
Capsaicin (NGX-3781, TQ-1018)	Pre-registered	Neuropathic pain and diabetic neuropathy	Multitargeted inhibitor, especially against NF-κB, TRPV1 and tNOX	NeurogesX
Vicoprofen® (hydrocodone/ibuprofen)[a]	Launched 2003	Nonspecific inflammation	NF-κB and COX-1/2/3 inhibitor	Abbott
Salsalate (NSC-49171)	Launched 1993	Rheumatoid arthritis, diabetes, osteoarthritis	NF-κB targeted inhibitor	Roche

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Table I. Contd

Compound	Development phase	Therapeutic targets	Mechanism of action	Originator(s)
Rhinadvil® (pseudoephedrine/ibuprofen)[a]	Launched 2005	Upper respiratory tract disorders	NF-κB and COX-1/2/3 inhibitor	SCOLR Pharma
Iguratimod (T-614)	Pre-registered	Rheumatoid arthritis	NF-κB targeted inhibitor	Toyama
Curcumin (diferuloylmethane)	Phase III	Genetic ocular disorders, arthritis, Alzheimer's disease, psoriasis, cystic fibrosis, premalignant conditions, malaria, myelodysplastic syndrome, pancreatic cancer, multiple myeloma, mucositis	Multitargeted inhibitor, especially against NF-κB, EGFR and cyclin D1 expression, glucose-6-phosphatase, HIV integrase and COX-2	Johns Hopkins University
Flurbiprofen (MPC-7869, E-7869)	Phase III	Nonspecific inflammation, arthritis, Alzheimer's disease, colorectal and prostate cancers	NF-κB modulator and γ-secretase inhibitor	Loma Linda University and Encore
Dimethyl fumarate (BG-12, AZL-O-211089)	Phase III	Psoriasis and multiple sclerosis	NF-κB targeted inhibitor	Biogen Idec
HZT-501 (famotidine/ibuprofen)[a]	Phase III	Nonspecific inflammation	Multitargeted inhibitor, especially against NF-κB and COX-1/2/3. Histamine H_2 receptor antagonist	Horizon Therapeutics
Fluasterone	Phase II	Psoriasis, arthritis, systemic lupus erythematosus, diabetes, actinic keratoses, multiple sclerosis	NF-κB targeted inhibitor	Temple University
Tepoxalin (Orf-20485, RWJ-20485)	Phase II	Psoriasis, ophthalmic inflammation, allergy, asthma	NF-κB and COX-1/2/3 inhibitor	Ortho-McNeil
E-3330	Phase II	Nonspecific inflammation	NF-κB targeted inhibitor	Eisai
Epigallocatechin gallate (EGCG)	Phase II	Actinic keratoses, lipoprotein disorders, dermatological disease, diabetes, ophthalmic disorders, Parkinson's disease, cancers and liver fibrosis	Multitargeted inhibitor, especially against NF-κB, SGLT-1, PDGFR, BACE, VEGFR, VEGFR-2 (FLK-1/KDR), tNOX and AP-1	Kyushu University
Declopramide (chloroprocainamide, 3-CPA)	Phase II	Inflammatory bowel disease and colorectal cancer	NF-κB targeted inhibitor	OxiGene
Resveratrol (SRT-501, RM-1812)	Phase II	Psoriasis, ocular disorders, diabetes, disorders of the coronary arteries and atherosclerosis, obesity, herpes virus infection, genetic neuromuscular disorders	Multitargeted inhibitor, especially against NF-κB, COX-1, xanthine oxidase, MAO-A and BACE-1. ApoA1 expression enhancer and SIRT1 activator	Royalmount Pharma
SMS-113 (tranexamic acid/ibuprofen)[a]	Phase II	Nonspecific inflammation	NF-κB and COX-1/2/3 inhibitor	Sawai

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Table I. Contd

Compound	Development phase	Therapeutic targets	Mechanism of action	Originator(s)
CDDO-Me (RTA-402, TP-155C)	Phase I/II	Inflammatory bowel disease, autoimmune diseases, rheumatoid arthritis, melanoma, solid tumours, renal diseases, pancreatic cancer	Multitargeted inhibitor, especially against NF-κB, Bcl-2, IKK-1 (IKK-α) and NOX production. PPARγ receptor agonist, NADPH and HO activator	Dartmouth College and MD Anderson Cancer Center
Orazipone (OR-1384)	Phase I	Inflammatory bowel disease, allergy, asthma	NF-κB targeted inhibitor	Orion
CDDO (RTA-401, NSC-711193, TP-151)	Phase I	Inflammatory bowel disease, stroke, solid tumours, leukaemia	Multitargeted inhibitor, particularly against NF-κB and NOX production. PPARγ receptor agonist	Dartmouth College and National Cancer Institute (US)
Betulinic acid (ALS-357)	Phase I	Melanoma and SARS	Multitargeted inhibitor, particularly against NF-κB, DGAT, SARS coronavirus 3C-like protease and DNA topoisomerase-I. Caspase-3/8 activator	University of Illinois
IMD-0354	Phase I	Interstitial lung diseases, disorders of the coronary arteries and atherosclerosis, atopic dermatitis	NF-κB and IKK-2 (IKK-β) inhibitor	Institute of Medicinal Molecular Design
SIM-688 (WAY-204688)	Phase I	Rheumatoid arthritis, nonspecific inflammation, sepsis	NF-κB inhibitor and estrogen receptor α/β ligand	Wyeth Pharmaceuticals
ENMD-1198	Phase I	Nonspecific inflammation and solid tumours	Multitargeted inhibitor, particularly against NF-κB, STAT-3 and HIF-1α factors	EntreMed
Parthenolide (NSC-157035)	Clinical	Atherosclerosis, leishmaniasis, septic shock	NF-κB targeted inhibitor	Ashbury Biologicals
Cepharanthine (NSC-623442)	Clinical	Nonspecific inflammation, HIV infection, cancers	NF-κB targeted inhibitor	Tohoku Pharmaceutical University
Taxifolin (dihydroquercetin [diquertin], distylin)	Clinical	Atherosclerosis, lipoprotein disorders, ischaemic stroke, hepatitis virus, HIV infection	Multitargeted inhibitor, especially against NF-κB, HMG-CoA reductase, reverse transcriptase and ApoB secretion	Sigma Chemical and National Yang-Ming University
SC-514	Early clinical trials	Nonspecific inflammation and anti-tumour promoting effects, particularly against adenocarcinoma	Selective inhibitor of IKK-2 activity	Pfizer
TPCA-1	Early clinical trials	Corneal ulcer, COPD and related airways inflammation	Inhibitor of human IKK-2 activity	GlaxoSmithKline
BMS-345541	Early clinical trials	Lung inflammation including airways inflammation in asthma, arthritis, inflammatory bowel diseases and cancer. Also suppresses graft rejection	Highly selective and potent inhibitor of IKK-2 activity. Binds to an allosteric site	Bristol-Myers Squibb

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Table I. Contd

Compound	Development phase	Therapeutic targets	Mechanism of action	Originator(s)
ML120B	Early clinical trials	Rheumatoid arthritis, COPD (particularly chronic airways inflammation) and cancer	Selective, reversible and ATP-competitive small-molecule inhibitor of IKK-β	Millennium Pharmaceuticals

ApoA1 = apolipoprotein A1; **ApoB** = apolipoprotein B; **BACE** = β-site amyloid precursor protein-cleaving enzyme; **COPD** = chronic obstructive pulmonary disease; **DGAT** = diacylglycerol acyltransferase; **FLK-1** = fetal liver kinase-1; **HMG-CoA** = hydroxymethylglutaryl coenzyme A; **HIF** = hypoxia-inducible factor; **KDR** = kinase insert domain receptor; **MAO** = monoamine oxidase; **NADPH** = nicotinamide adenine dinucleotide phosphate; **NOX** = nicotinamide adenine dinucleotide oxidase; **OCD** = obsessive-compulsive disorder; **PPAR** = peroxisome proliferator activated receptor; **SARS** = severe acute respiratory syndrome; **SGLTI** = sodium-dependent glucose transporter; **SIRT1** = silent mating type information regulation 2 homologue *Saccharomyces cerevisiae*; **tNOX** = tumour-associated nicotinamide adenine dinucleotide oxidase. For all other abbreviations, please refer to text.

has also been found that cysteinyl leukotrienes can directly activate the NF-κB signalling pathway.[25,26] Due to this mechanism of action, pranlukast strongly inhibits NF-κB signalling pathways with subsequent reductions in the growth factor regulated on activation, normal T cell expressed and secreted (RANTES), transforming growth factor (TGF)-β, T helper 2 (T_h2) cells, IL-4/6/11/13, IFNγ and vascular endothelial growth factor (VEGF) gene expression, and in LPS production and activity, which in turn might cause migration of eosinophils and activated T-lymphocytes into the inflammatory nidus. However, a series of recent studies have strongly suggested that pranlukast also inhibits the NF-κB signalling route and expression of related pro-inflammatory genes including IL-5, mucin 2 (oligomeric mucus/gel-forming) [MUC2] and TNFα via a mechanism distinct from antagonism at leukotriene receptors.[27-29]

Gabexate

Gabexate is an effective NF-κB, AP-1 and protease inhibitor that was launched in 1978 by Ono (Osaka, Japan) as a powder for injection for the treatment of disseminated intravascular coagulation and acute pancreatitis. Originally developed at Ono, the drug was later licensed to Lepetit. In particular, this compound was considered to be a key mediator in the regulation of the immune response to infection, inflammatory-related diseases including cancer and autoimmune disorders, septic shock, viral penetration and improper immune development. For example, in human pancreatic cancer cell lines, gabexate strongly inhibits TNFα-induced NF-κB activation and enhances cellular apoptosis, mainly through activation of caspases 3 and 7, and dramatically suppresses the invasive potential of cells studied.[30] This drug has also been shown to reduce endotoxin-induced pulmonary vascular injury in an animal model of sepsis by inhibiting leukocyte activation.[31] Gabexate also inhibits the expression of leukocyte adhesion molecules by blocking the NF-κB-mediated transcription in human umbilical vein endothelial cells (HUVECs).[32] Presumably, the actual mechanism of action is based on inhibition of IκB degradation, resulting in suppression of TNFα production in monocytes stimulated by LPS. In addition, gabexate might significantly reduce LPS-induced pulmonary vascular injury not only by inhibiting monocytic TNFα production but also by inhibiting the expression of endothelial leukocyte adhesion molecules.[33]

Dimethyl Fumarate

Dimethyl fumarate (DMF), an NF-κB activation inhibitor, is currently undergoing phase III clinical trials at Biogen Idec (Cambridge, MA) for the treatment of relapsing-remitting multiple sclerosis. The company had also been investigating this compound for the oral treatment of mild to moderate psoriasis. DMF is a second-generation fumarate derivative with promising anti-inflammatory potential. An early study showed that DMF potently inhibits ICAM-1, VCAM-1 and E-selectin expression as well as reduces adhesion of U937 cells to stimulated HUVECs through inhibition of the NF-κB signalling pathway.[34] It was also found that DMF effectively blocks the TNF-induced translocation of NF-

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κB/p65 transcriptional complex into the nucleus of human endothelial cells.[35] Recent studies provide a detailed profile of DMF action. It has been shown that DMF inhibits nuclear entry of NF-κB in rat heart endothelial cells and significantly reduces myocardial infarct size after ischaemia and reperfusion in rats *in vivo*.[36] A recent study demonstrates that DMF can effectively prevent the TNFα-induced increase in macrophage colony-stimulating factor (M-CSF) production by inhibiting NF-κB transcriptional activity.[37] DMF was found to significantly inhibit both mitogen and stress-activated (MSK)-1 and MSK2 activities in cultured human keratinocytes.[38] Also, this study provided strong evidence that DMF decreases phosphorylation of NF-κB/p65 complex, which is known to be transactivated by MSK1. Furthermore, a significant decrease in NF-κB binding to the IL-8/20 DNA domains, leading to a related decrease in IL-8 and IL-20 mRNA expression, was also estimated. The results obtained strongly suggest that DMF specifically inhibits MSK1/2 activations and subsequently blocks NF-κB-induced gene transcriptions, which are extremely important in the pathogenesis of inflammation. Another study highlighted the fact that the suppressive effect of DMF on pro-inflammatory cytokine and chemokine production is directly associated with selective inhibition of the NF-κB signalling pathway.[39] In addition, DMF was shown to induce apoptosis in various cells, particularly human T cells.[40]

Curcumin and Resveratrol

At least two well known plant-derived flavonoids, curcumin and resveratrol, have been found to be key mediators of the NF-κB signalling pathway.[41] A wealth of data gathered over the past 15 years of intensive research in this field strongly suggest that these two agents are leaders in the field of potent anti-inflammatory therapeutics.[42]

Curcumin: Curcumin, a polyphenol present in the spice turmeric, can directly scavenge free radicals such as superoxide anion and nitric oxide and modulate important signalling cascades mediated via NF-κB, MAPK and JAK/STAT pathways.[43] This compound is in phase III clinical development against various inflammatory diseases, including fibrosis, asthma and chronic obstructive pulmonary disease.[44,45] Curcumin downregulates the expression of many pro-inflammatory mediators and specific proteins, including cytokines, MMPs, adhesion molecules and GF-R genes.[46-49] In general, curcumin acts by inhibiting phosphorylation of IκB, which in turn reduces nuclear translocation of NF-κB. This mechanism of action is strongly associated with decreased production of the pro-inflammatory chemokines CXCL1, CXCL2 and CXCL8 as well as the chemokine receptor CXCR4.[50,51] In addition to acting through the typical NF-κB pathway, curcumin was found to strongly modulate p38 MAPK, STAT-3 and JNK signalling cascades, mainly through blockade of TNFα precursor activity in HUVECs.[52] It was also estimated that expression of ICAM-1, MCP-1[53] and IL-8 can be slightly reduced by curcumin; however, curcumin does not affect expression of TNFR I and II. Similar effects were observed for chalcone, an α,β-unsaturated flavonoid with promising anti-inflammatory properties.[54] It has been concluded that orally ingested curcumin reverses many of the inflammatory and metabolic derangements associated with obesity and improves glycaemic control in mouse models of type 2 diabetes mellitus.[55] The observed effect was strongly associated with perturbations in NF-κB signalling.

It has also been shown that curcumin potently inhibits both NF-κB and STAT-3 activities in Hodgkin-Reed Sternberg cells, leading to decreased expression of proteins involved in cell proliferation and apoptosis, e.g. B-cell lymphoma-2 (Bcl-2), basal cell lymphoma-extra large (Bcl-xL), cellular caspase-8 (Fas associated protein with death domain-like IL-1β-converting enzyme)-like inhibitory protein (cFLIP), X-linked inhibitor of apoptosis protein (XIAP), cellular inhibitor of apoptosis 1 protein (c-IAP1), survivin, c-Myc and cyclin D1.[56] Furthermore, curcumin triggers cell death by apoptosis, as evidenced by activation of caspases 3 and 9, changes in nuclear morphology and phosphatidylserine translocation. Curcumin also suppresses both ligand-induced and lauric acid-induced Nod2 sig-

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nalling, which results in suppression of NF-κB activation and target gene IL-8 expression.[57] Chen et al.[58] have shown that interruption of NF-κB and extracellular signal-regulated kinase (ERK) signalling by curcumin may result in the complete suppression of connective tissue growth factor expression in activated hepatic stellate cells *in vitro*.

Curcumin has also been shown to effectively prevent the degradation of IκB-α, which in turn inhibits α-lipoic acid (ALA)-mediated activation of NF-κB and related upregulation of MMP-9.[59,60] These results strongly indicate that in peripheral blood mononuclear cells (PBMCs) the anti-inflammatory effect of curcumin involves inhibition of production of MMP-9. It has also been demonstrated that curcumin downregulates P-glycoprotein (P-gp) expression, encoded by the multiple drug resistance (MDR) gene, in multidrug-resistant L1210/Adr cells, presumably through inhibition of the PI3K/Akt/NF-κB signalling pathway.[61] The spectrum of the anti-inflammatory action attributed to curcumin is not restricted solely by the activities mentioned above. A large number of scientific papers published during the last decade highlight the prominent role of curcumin-based treatment of various chronic inflammatory diseases.[62]

Resveratrol: At the end of the 1990s, resveratrol began to receive considerable attention as a novel promising anti-inflammatory agent that specifically acts against NF-κB signalling pathways. In 1999, Wadsworth and Koop[63] showed that resveratrol inhibited production of TNFα by blocking LPS-stimulated activation of the NF-κB route. Subsequent analysis has clearly shown that resveratrol inhibits the phosphorylation as well as degradation of IκB-α, which hampers nuclear translocation of the transcriptionally active subunits of NF-κB.[64-66] Subsequently, Pellegatta et al.[67] speculated that instead of serine phosphorylation, resveratrol increases tyrosine phosphorylation of IκB-α, p50/NF-κB and p65/NF-κB, suggesting the involvement of such alterations in the modulation of NF-κB transcription activity. Similar anti-inflammatory action has also been attributed to piceatannol, a stilbene that is structurally homologous to resveratrol.[68] It has also been observed in lymphoid (Jurkat) and epithelial (HeLa and H4) cells that resveratrol significantly inhibits NF-κB activation induced by different stimuli.[69] Suppression of NF-κB was accompanied by a decrease in AP-1 activity. Resveratrol also inhibited the TNF-induced activation of MAPKs and c-Jun kinase as well as abrogated TNF-induced cytotoxicity and caspase activation. In addition, both resveratrol and quercetin have been suggested to have a powerful nonsteroidal anti-inflammatory activity that may have applications in the treatment of inflammatory diseases.[70]

During the past 5 years, the anti-inflammatory activity of resveratrol has been vigorously described in numerous scientific papers. Several recent studies have revealed that resveratrol significantly suppresses LPS-induced degradation of IκB-α guardian complexes, expression of iNOS and phosphorylation of p38 MAPK in N9 microglial cells.[71,72] Experiments performed in severe acute pancreatitis rats have clearly indicated that activation of NF-κB is deeply involved in the related inflammatory response.[73] Resveratrol was found to effectively inhibit the expression of NF-κB activation, alleviate the severity of severe acute pancreatitis, and regulate the activity of various inflammatory mediators.

Gene analysis has also clearly shown that resveratrol can entirely suppress expression of various pro-inflammatory factors, including ICAM-1, RANTES and MCP-1 proteins, TNF receptor superfamily (TNFRSF) and TLR3 receptors, pro-inflammatory cytokines that attract monocyte-granulocyte cells such as M-CSF, granulocyte-macrophage CSF (GM-CSF) and granulocyte CSF (G-CSF) as well as TGFβ and IL-1α through inhibition of the NF-κB signalling pathway.[74] The NF-κB-associated mechanisms of anti-inflammatory action of resveratrol have been comprehensively reviewed by de la Lastra and Villegas.[75] Interestingly, resveratrol was also found to reduce LPS-induced airway inflammation,[76] but the reduction does not appear to be due to an impact on NF-κB activation or the expression of the respective genes, as suggested by various *in vitro* studies.[77] The results obtained strongly suggest that resveratrol may exert a benefi-

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cial anti-inflammatory effect via a novel mechanism of action. More recently, resveratrol was found to significantly suppress NF-κB activation and COX-2 expression in RAW 264.7 cells following TLR3 and TLR4 stimulation, but not stimulation of TLR2 or TLR9.[78] Recently, Zhu et al.[79] have confirmed the declared ability of resveratrol to inhibit DNA-binding activity of the NF-κB complex and subsequently suppress TNFα-induced MCP-1 gene expression.

SC-514

SC-514 is a selective inhibitor of IKK-2 activity of the native IKK complex or the recombinant human IKK-1/IKK-2 heterodimer. It is especially important to note that this compound does not inhibit other IKK isoforms or other serine-threonine and tyrosine kinases. The inhibition is selective, reversible and competitive with ATP molecules. In particular, SC-514 inhibits transcription of NF-κB-dependent genes in IL-1β-induced RA-derived synovial fibroblasts in a dose-dependent manner.[80] It was found that SC-514 did not inhibit phosphorylation and further activation of the IKK complex directly. Thus, it was suggested that the effect of SC-514 on cytokine gene expression and corresponding pro-inflammatory mediator production is largely the product of the combination of inhibition of IκB-α phosphorylation/degradation, affecting NF-κB nuclear import/export, and phosphorylation and transactivation of the p65 unit.[80] The perturbations in the NF-κB signalling pathway lead to attenuation of nitric oxide (NO), CXCL2 and TNFα release, the last of these being a pivotal precursor for augmenting TLR2 and IL-6 expression.[81] Thus, in human adipocytes, it was found that IL-6 release stimulated by thyroid-stimulating hormone was 60% inhibited by SC-514.[82] SC-514 was also found to strongly inhibit IKK-β activity and related TNFα-induced activation of NF-κB as well as TNFα-promoted metastasis of murine colon adenocarcinoma cells, particularly through the blocking of MMP-9 enzymatic activity.[83] In mice models, SC-514 1 μmol/L significantly attenuated tissue plasminogen activator (TPA)-induced activation of Akt and NF-κB, and also the expression of COX-2 in hairless mouse skin.[84] Rational use of SC-514 has been reported in a therapeutic combination with 9Z,11E-CLA (cis-9,trans-11-conjugated linoleic acid).[84] This drug combination leads to a drastic inhibition of NF-κB-driven-COX-2 expression by blocking IKK and PI3K-Akt signalling in TPA-treated hairless mouse skin *in vivo*, which may account for the previously reported anti-tumour-promoting effects of the combination. In RAW 264.7 murine macrophage-like cells stimulated with LPS, SC-514 was found to inhibit LPS-induced NF-κB DNA binding.[85] However, SC-514 does not inhibit LPS-induced secretion of HMGB1, a nuclear protein that is secreted by immunostimulated macrophages and acts as a key pro-inflammatory mediator. Pretreatment of rat aortic smooth muscle cells with SC-514 significantly reduced iNOS induction, NF-κB translocation and IκB-α loss in a concentration-dependent manner.[86] It was also found that SC-514 and BMS-345541 (see below) significantly blocked IgE/trinitrophenyl (TNP)-induced TNF production by mouse bone marrow-derived mast cells (BMMC).[87] In turn, IgE and TNP jointly induce a rapid phosphorylation of IKK-α but not IKK-β in BMMC. This was accompanied by phosphorylation and degradation of IκBα, subsequent NF-κB activation, and TNF production. Interestingly, IgE/TNP-induced IKK-α and IκBα phosphorylation were completely inhibited by a protein kinase C (PKC) inhibitor, Ro-31-8220. These results highlight a role of the IKK-IκB-NF-κB pathway in the regulation of mast cell function in allergy.

TPCA-1

TPCA-1 is a novel, highly potent (concentration that produces 50% inhibition [IC_{50}] = 17.9 nmol/L) and selective small-molecule inhibitor of human IKK-2 activity.[88] This compound inhibits LPS-induced human monocyte production of several pro-inflammatory mediators, including TNFα, IL-6 and IL-8 with IC_{50} values in the range of 170–320 nmol/L. In mice with collagen-induced arthritis, TPCA-1 administration at a dosage of 3, 10 or 20 mg/kg (intraperitoneally, twice daily) led to reductions and delays in disease severity.[88] The maximum therapeutic action was observed at 20 mg/kg. It was found that nuclear localization of p65, as well as

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levels of IL-1β, IL-6, TNFα and IFNγ were significantly reduced in the paw tissue of TPCA-1- and etanercept-treated mice. In addition, administration of TPCA-1 *in vivo* resulted in significantly decreased collagen-induced T-cell proliferation *ex vivo*. The therapeutic potential of TPCA-1 against corneal ulcer caused by collagen degradation in corneal stroma was recently investigated by Kondo et al.[89] These investigators found that IL-1β-induced collagen degradation was powerfully inhibited by TPCA-1 in a concentration- and time-dependent manner through IKK-2 blockage. TPCA-1 inhibited both the IL-1β-induced expression of MMP-1, -3 and -9 in the cells studied at both the mRNA and protein levels as well as IL-1β-induced activation of pro-MMP-2. In contrast to dexamethasone, TPCA-1 prevents the phosphorylation and degradation of IκB-α as well as the nuclear translocation of NF-κB.

Since the severity of chronic obstructive pulmonary disease (COPD) strongly correlates with increased numbers of cytotoxic CD8+ T-lymphocytes in the lung parenchyma, which in turn stimulate IFNγ release leading to chemokine receptor CXCR3 production in airway epithelial cells, TPCA-1 in its role as a strong inhibitor of CXCL9, CXCL10 and CXCL11 release can be reasonably regarded as a potential therapeutic agent against COPD. Indeed, in human bronchial epithelial cell line BEAS-2B stimulated by IFNγ, TPCA-1 was found to inhibit the listed chemokine activities by reducing the IκB kinase-2 activity with half maximally effective concentration (EC_{50}) values of 0.50, 0.17 and 0.45 μmol/L, respectively, in regard to CXCL9, CXCL10 and CXCL11.[90]

In the LPS model of airway inflammation, TPCA-1 was found to significantly block the increase in NF-κB DNA binding.[91] This inhibition was directly associated with a reduction in inflammatory mediator release, principally for TNFα, IL-1β, MMP-9 and in lung inflammatory cell burden (neutrophilia/eosinophilia). This was the first study to examine the effect of an IKK-2 inhibitor in well validated models that mimic aspects of the inflammatory lesion evident in diseases such as COPD. The investigators have demonstrated that animal models with similar profiles of airway inflammation can be IKK-2 inhibitor/corticosteroid-sensitive or -insensitive; both profiles of inflammation are widely distributed in the clinic. Therefore, this finding is extremely exciting and may lead to greater understanding of disease pathology and the discovery of novel anti-inflammatory targets. In addition, Birrell and coworkers[92] soon afterwards reported that the therapeutic impact of TPCA-1 (in rats) can be effectively monitored using exhaled nitric oxide, which has been shown to be a reproducible, noninvasive indicator of the inflammatory status of the airway in the clinic.

BMS-345541

BMS-345541 is a highly selective and potent inhibitor of IKK-2 (IC_{50} = 0.30 μmol/L), but considerably less potent against IKK-1 (IC_{50} = 4.0 μmol/L).[93] It should be especially noted that BMS-345541 was the first example of an inhibitor of IκB kinase with anti-inflammatory activity *in vivo*. In cell models, BMS-345541 failed to inhibit a set of 15 other kinases, including c-Jun and STAT-3, as well as MAPK-activated protein kinase-2 (MAPKAPK-2).[94] Consistent with the role of IKK/NF-κB in the regulation of pro-inflammatory cytokine production, BMS-345541 inhibited LPS-stimulated TNFα, IL-1β, IL-8 and IL-6 release in THP-1 (human acute monocytic leukaemia) cells with IC_{50} values in the 1–5 μmol/L range. The constructed binding model shows that BMS-345541 binds to similar allosteric sites located in both the IKK-1 and IKK-2 catalytic complexes, which then affects the active sites of the subunits differently. BMS-345541 has also been shown to have excellent pharmacokinetics in mice, and oral administration showed that the compound dose dependently inhibits the production of serum TNFα following intraperitoneal challenge with LPS. Thus, the compound is effective against NF-κB activation in mice and represents an important tool for investigating the role of IKK in disease models. It has also been found that BMS-345541 inhibits IL-6 and COX-2 production and activity with reduction in PGE_2 level in LPS/TLR4-stimulated chicken thrombocytes *in vitro*.[95]

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Furthermore, it has previously been shown that BMS-345541 blocks both inflammation and joint destruction in murine collagen-induced arthritis.[96]

BMS-345541 has also been tested in mice for its ability to inhibit anti-CD3-induced IL-2 and TNFα production, as well as T-cell proliferation, in an *in vivo* mixed lymphocyte reaction, and to suppress graft rejection in a murine nonvascularized heterotopic cardiac allograft model.[97] The compound was tested as a single agent and in combination with other immunomodulators for inhibition of T-cell proliferation and graft rejection *in vivo*. The best therapeutic outcome was achieved when the drug was combined with cytotoxic T-lymphocyte antigen-4 immunoglobulin. It was found that at a dose of 100 mg/kg BMS-345541 suppressed the production of both IL-2 and TNFα (by up to 70%) in mice stimulated with an injection of anti-CD3 antibody in a dose-dependent manner, resulting in 77% inhibition of CD4+ T-cell proliferation.

Immune complexes are key players in a variety of autoimmune diseases: they stimulate monocyte recruitment through interaction with immunoglobulin constant fragment (Fc)γ-receptor (a high affinity receptor for IgG), triggering the secretion of several pro-inflammatory mediators and favouring their tissue accumulation by inhibiting apoptosis. BMS-345541 was found to abolish the apoptosis protection conferred by immune complexes by inhibiting IKK complexes, thereby preventing the progress of inflammation.[98] The same therapeutic effect of BMS-345541 has also been reported in relation to IFNγ-stimulated macrophage recruitment. Specifically, it has been clearly shown that IFNγ induction of Fcγ upregulation is strongly dependent on the IKK/NF-κB pathway.[99]

Besides playing a pivotal role in cytoprotection and inflammation, TNFα is also regarded as a key necrotic death precursor in tumours *in vivo*. BMS-345541 has been found to increase TNFα killing in TNFα-resistant tumour cell lines by increasing cellular apoptosis, a finding that suggests that in addition to inflammation, inhibition of NF-κB could be an effective strategy for enhancing the tumoricidal effects of TNFα.[100]

It was recently disclosed that airway smooth muscle cells can act as effector cells towards the initiation and/or perpetuation of airway inflammation in asthma by producing various pro-inflammatory mediators, such as IL-6, IL-8 and eotaxin.[101] This action is accompanied by enhanced secretion of TNFα and IFNγ or endogenous IFNβ, resulting in a synergistic induction of various pro-inflammatory genes, including CD38. These genes were also found to be NF-κB-dependent in that TNFα-induced expression of IL-6, IL-8 and eotaxin was dose dependently inhibited by BMS-345541 1–30 μmol/L.[102]

It was recently suggested that NF-κB-dependent expression of MMP-9 in response to cytosine-phosphate-guanine oligodeoxynucleotide (CpG-OGN) plays an important role in the recruitment of immune cells into the inflammation nidus.[103] In particular, an MMP-9 precursor was activated by the stimulation of CpG-ODN and ectopic expression of NF-κB transcription factor. BMS-345541 was found to dramatically inhibit the expression of the *MMP-9* gene induced by CpG-ODN.[103] In addition, in SW-1353 human chondrosarcoma cells, BMS-345541 inhibited IL-1-dependent expression of MMP-1, MMP-3 and MMP-13 in a concentration-dependent manner, thereby blocking aggrecan and collagen degradation.[104]

BMS-345541 has also been used to determine whether intervention in the NF-κB pathway could prevent progression to lung injury in the LPS pump model.[105] The compound was administered into the model cells to downregulate NF-κB activation following the onset of inflammation. Treatment with BMS-345541 significantly reduced lung NF-κB activation, concentration of the chemokine KC and macrophage-inflammatory protein (MIP)-2 in lung lavage, neutrophil influx and lung oedema. Therefore, it was suggested that sustained NF-κB activation strongly correlates with severity of lung injury, and that blockage of the NF-κB pathway by BMS-345541 is quite beneficial even after the onset of lung inflammation.[105]

Inflammatory bowel diseases, including ulcerative colitis and Crohn's disease, are frequently ac-

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companied by chronic relapsing inflammation supported by the transcription of various pro-inflammatory mediators that regulate pathogenesis in inflammatory bowel disease (e.g. TNFα, ICAM-1 and VCAM-1). Such expression is meticulously maintained by the NF-κB signalling system activated via IκB kinase. It has been shown that BMS-345541 strongly inhibits TNFα-induced expression of both ICAM-1 and VCAM-1 in HUVECs with an IC_{50} of 5 μmol/L.[106] BMS-345541 administered orally at doses of 30 and 100 mg/kg was effective in blocking both clinical and histological endpoints of inflammation and injury. These findings clearly indicate that inhibitors of IκB kinase activity may be highly efficacious in inflammatory disorders such as inflammatory bowel disease.

Finally, BMS-345541 has also been evaluated in collagen-induced arthritis in mice.[107] BMS-345541 10–100 mg/kg showed exciting potential in reducing the incidence of disease and inhibiting the clinical signs of collagen-induced arthritis. Histological evaluation of the joints showed that both inflammation and joint destruction were effectively blocked by BMS-345541.

ML120B

ML120B is a potent, selective, reversible and ATP-competitive small-molecule inhibitor of IKK-β with an IC_{50} of 60 nmol/L (IκB-α kinase complex assay).[108] ML120B does not inhibit other IKK isoforms or a panel of other kinases. This compound strongly inhibits TNFα-stimulated NF-κB signalling via inhibition of the target IκB-α phosphorylation, degradation and NF-κB translocation into the nucleus.[109] As mentioned above, the IKK/NF-κB signalling pathway is deeply implicated in the RA disease process. For example, in rats, oral administration of ML120B inhibited paw swelling in a dose-dependent manner, thereby leading to significant protection against RA-induced weight loss as well as cartilage and bone erosion.[110] NF-κB activity in arthritic joints reduced dramatically after ML120B administration. In addition, it was observed that downregulation of the IKK-β/NF-κB signalling pathway resulted in significant inhibition of the chronic inflammatory process associated with rat adjuvant-induced arthritis. Similar results were also obtained in the murine model of antibody-induced arthritis.[111] ML120B blocked numerous NF-κB-regulated cell responses involved in inflammation and destructive processes in the RA joint. *In vivo* imaging demonstrated that NF-κB activity in inflamed arthritic paws was strongly inhibited by ML120B, resulting in significant suppression of several pro-inflammatory genes, including KC (murine IL-8), epithelial neutrophil-activating peptide 78, JE, ICAM-1, CD3, CD68, TNFα, IL-1β, IL-6, iNOS, COX-2, MMP-3, cathepsin B and cathepsin K. It was also found that in mice, ML120B sensitized bone marrow progenitors and granulocytes and induced cellular apoptosis in the bone marrow and spleen after oral administration.[112] In particular, inhibition of IKK-β by ML120B resulted in depletion of thymocytes and B cells in all stages of development in the bone marrow but did not deplete granulocytes. ML120B-based therapy also leads to rapid TNF-dependent depletion of T and B cells. This observation has several important implications for the potential use of ML120B in the treatment of inflammatory disease and cancer.

Asthma and COPD are characterized by chronic airway inflammation and the airway epithelium is critical in the pathogenesis of these chronic inflammatory diseases. Chronic airway inflammation is invariably accompanied by the expression of various pro-inflammatory genes associated with the NF-κB signalling route. Accordingly, ML120B was evaluated in two cell models: human A549 pulmonary cells and primary human bronchial epithelial cells.[113] In the first system, IL-1β and TNFα significantly increased phosphorylation of IκB-α, and this was followed by loss of IκB-α, induction of NF-κB DNA binding, and the induction of NF-κB-dependent transcription. The observed activity was strongly inhibited by ML120B in a dose-dependent manner, resulting in loss of ICAM-1 expression. Similarly, IL-1β - and TNFα-induced expression of IL-6, IL-8, GM-CSF, RANTES, growth-regulated oncogene (GRO)-α and MCP-1 were also significantly repressed. Similar results were previously obtained by Catley and coworkers.[114]

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1.2 P38 Mitogen-Activated Protein Kinase (MAPK) Signalling Pathway

It is now abundantly clear that MAPKs play an essential regulatory role in the production of various pro-inflammatory factors deeply implicated in inflammation and that they strongly regulate critical pro-inflammatory signalling cascades and cytokine biosynthesis at the transcriptional and translational levels. For these reasons, MAPKs provide a potential therapeutic target for prevention of different inflammatory conditions. This signalling system is currently considered to be a crucial element in the induction and maintenance of acute and chronic inflammation, and its components thus emerge as interesting molecular targets for small-molecule inhibitors. Several classes of mammalian MAPKs have been identified to date, including ERK, JNK and p38 MAPK. Although ERK pathways regulate cell survival and are responsive to extracellular mitogens, p38 MAPK and JNK are jointly involved in environmental stress responses, including many inflammatory stimuli. Among these kinases, p38 MAPK is a key intracellular mediator involved in the production of numerous pro-inflammatory factors such as TNFα and IL-6. A deeper understanding of the broad biological and pathophysiological roles of p38 MAPK family members has developed over the past decade, uncovering the inherent complexity of the signalling network leading to their activation. In addition to its effects on various growth factors, p38 MAPK regulates enzyme induction and transport, including COX-2, iNOS and MMPs, and controls adhesion molecule expression. Downstream substrates of MAPK include other kinases such as MAPKAPK-2, which is involved in the phosphorylation and activation of heat-shock proteins, and factors that regulate transcription, nuclear export and mRNA stability and translation.

Mammalian p38 MAPKs have been shown to be activated by a wide range of cellular stress conditions, including UV irradiation, heat shock and high osmotic stress, as well as in response to pro-inflammatory cytokines (such as IL-1 and TNFα), endotoxins, LPS, mitogens, protein synthesis inhibitors and specific mediators that exert their effect through interaction with different transmembrane receptors (figure 2). Membrane-bound receptors provide an essential link between the extracellular environment and the initiation of intracellular signalling events that activate common signalling components of the MAPK pathway. Four p38 MAPKs have been successfully cloned so far in higher eukaryotes: p38α/XMpk2/CSBP, p38β/p38-β22, p38γ/SAPK3/ERK6 and p38δ/SAPK4. These kinases are approximately 65% identical in their amino acid sequence but differ in their expression patterns, substrate specificities and sensitivities to chemical inhibitors such as SB-203580.

Activation of specific MAPKs involves highly regulated and modulated cascades of phosphorylation events mediated by sequential and concerted activation of upstream kinases. Although different MAPK cascades typically show high specificity and functional separation, some degree of cross-talk is clearly observed across different pathways. In general, ERKs are phosphorylated by members of the MEK family; JNK/SAPKs and p38 MAPKs are phosphorylated by SEKs (signal-regulated kinases) and MAPK kinases (MKKs). The MAP2Ks are phosphorylated by MAPK-kinase kinases (MAPKKK, MEKK or MAP3Ks), which are the key precursors of cell signals routed to specific MAPK cascades. A wide variety of kinases provide MAP3K function to the MAPK pathways. MAP3Ks, in turn, include the following members: TGFβ activated kinase 1 (TAK1), mixed lineage kinase (MLK), p21-guanosine triphosphate (GTP)-ase activated kinase (PAK), apoptosis signal-regulating kinase (ASK1) and B-Raf, and have been found to be activated by a vast range of signals initiated through different transmembrane receptors (figure 2). Downstream signals are typically transduced via guanine nucleotide exchange factors, which in turn activate small GTPases belonging to the Ras superfamily mainly composed of c/b-Raf, Rho, Rac, Rab and CDC42 proteins and specific kinases. The IL-1-receptors, TLRs, GPCRs and GPCR-like receptors, as well as epidermal growth factor receptors (EGFRs) and nerve growth factor receptors (NGFRs), trigger MAPK signalling cas-

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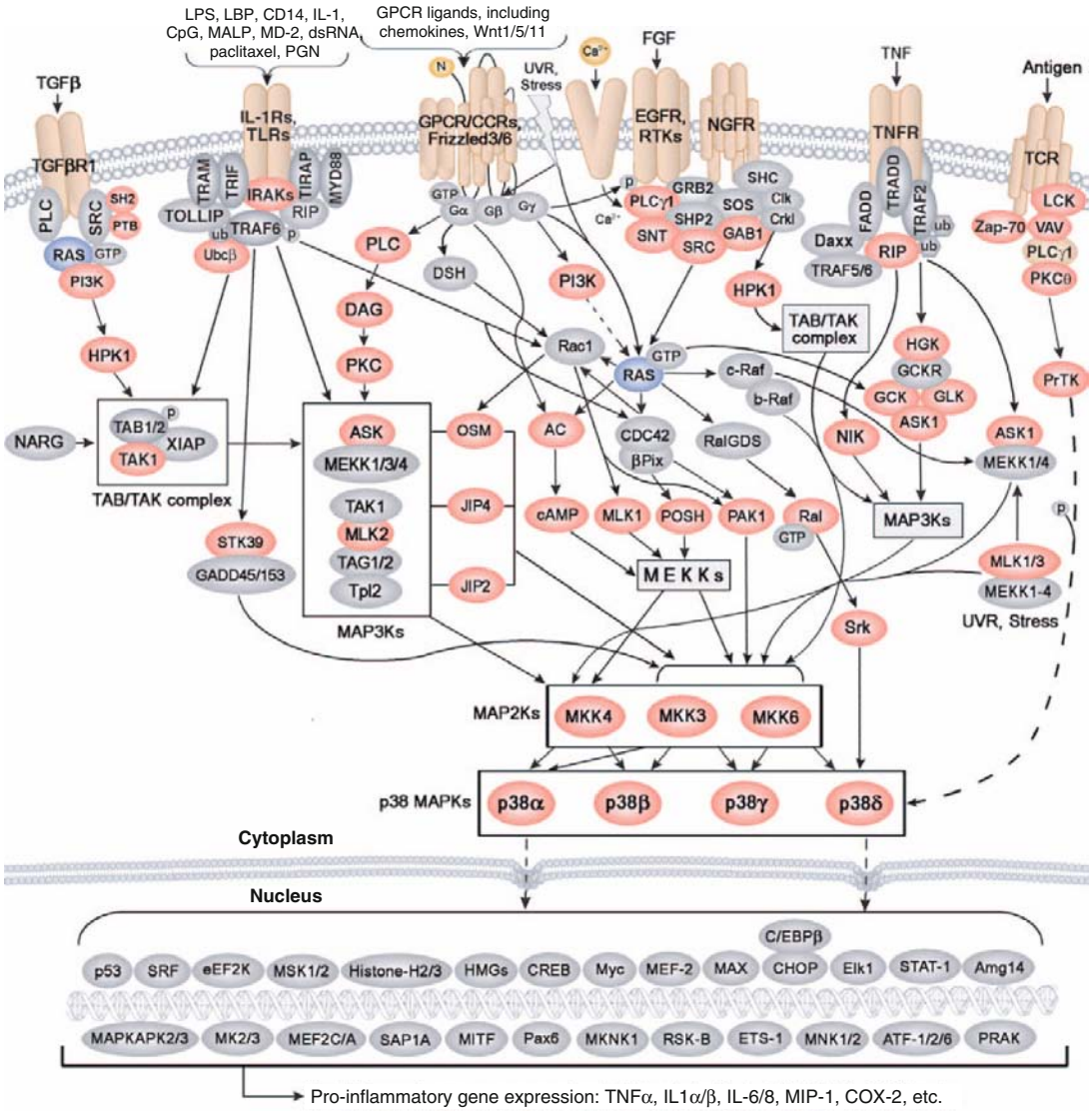

Fig. 2. Canonical and alternative nuclear transcription factor (NF-κB) signalling pathways implicated in inflammation. Please refer to text for definition of relevant abbreviations.

cades, leading importantly to translocation of p38 MAPKs, chiefly through activation of the Ras/GTPase system. The TNF/TNFR ligand-receptor pair directly activates the ASK1 complex, including hepatocyte progenitor kinase-like/germinal centre kinase-like kinase (HGK), glucokinase (hexokinase 4) regulator (GCKR), GCK and CDC-like kinase (CLK) subunits through TRAF2. ASK1 can also be activated by reactive oxygen species (ROS)-mediated dissociation of thioredoxin. Either of these routes can lead to subsequent activation of p38 MAPK-related transcription. TAK1 within the TAK1-binding subunit (TAB)-TAK-XIAP signalling complex mediates signal transduction from the TGFβ superfamily to p38 and ERK MAPKs via MAP3K activation. The core signalling scheme of

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p38 MAPK activation and transcription includes the following principal route: ligand/transmembrane receptor interaction; triggering of specific receptor-anchored or receptor-related proteins (e.g. GF-R-bound protein [GRB]-2, Src homologous and collagen-like protein [SHC], TRAP and TRAFs, phospholipase C [PLC], IL-1 receptor associated kinases [IRAKs], etc.); activation of the precursor system (e.g. ROS, TAK or ASK); MAP3Ks (MEKK 1-4) activation; MAP2Ks (MKK 3/4/6) activation; p38 MAPKs activation and translocation into the nucleus; recruitment of a large range of transcription factors and proteins (e.g. MSK1/2, STAT-1, myocyte enhancer factor [MEF]-1/2C/A, activating transcription factor [ATF]-1/2/6, MAPKAPK-2/3, etc.); and pro-inflammatory gene expression (TNFα, IL-1/6/8, MIP-1, COX-2, etc.).

1.2.1 P38 MAPK Inhibitors

Determination of the high-resolution crystal structure of p38α has recently led to the design of a number of small-molecule anti-inflammatory drug compounds with high pharmacological activity. The key therapeutic characteristics of the clinically proven small-molecule inhibitors of p38 MAPK are listed in table II and table III.

Cannabidiol

Cannabidiol (CBD), the main non-psychotropic component of *Cannabis sativa*, was recently reported to have a high anti-inflammatory activity, particularly against acute models of inflammation and diabetic retinopathy.[115] In this study, CBD treatment significantly reduced ROS production as well as decreased the levels of TNFα, VEGF and ICAM-1 *in vivo*. The observed effects were strongly associated with inhibition of p38 MAPK activity. A parallel study performed in PC12 cells clearly showed that CBD exhibits inhibitory activity mainly through inhibition of a phosphorylated form of p38 MAPKs and NF-κB activation in a concentration-dependent manner.[116] CBD exposure has also been shown to lead to a decrease in the levels of p38 MAPK in leukaemia cell lines.[117] In clinical practice, CBD is commonly used in combination with Δ9-tetrahydrocannabinol (THC) [Nabidiolex®], a potent agonist at cannabinoid receptors. Although THC activates cannabinoid CB2 receptors, thus leading to p38 MAPK activation, it has also been found to provide adequate protection against N-methyl-D-aspartic acid (NMDA)-induced apoptosis in AF5 cells by blocking ROS generation and inhibiting activation of p38-MAPKs.[118]

Chlomethiazole

Chlomethiazole and pyridinyl imidazole compounds, exemplified by SB-203580 (see next section), are structurally unrelated p38 MAPK inhibitors with promising anti-inflammatory activity and neuroprotective properties.[119] Northern blot analysis and quantitative real-time polymerase chain reaction techniques have shown that both compounds are highly potent suppressors of IL-1β-induced expression of c-Fos and iNOS.[120] In addition, chlormethiazole was found to selectively inhibit p38 MAPK activity in primary cortical glial cultures.[121]

SB-203580

Among all the p38 MAPK inhibitors currently evaluated in clinical trials, SB-203580 appears to be the most potent compound in terms of anti-inflammatory activity. A brief analysis of the scientific literature describing the huge therapeutic potential of SB-203580 in various inflammatory conditions revealed over 1000 publications in various high-profile journals. Because of space limitations we are focusing solely on advances that have been made since the beginning of 2008 towards SB-203580-based anti-inflammatory therapy. It was recently found that in dendritic cells SB-203580 significantly suppresses ERK1/2 inhibition induced by dinitrochlorobenzene (DNCB) or thiomersal, thereby demonstrating a direct interaction between p38 MAPK and ERK-1/2.[122] In human keratinocytes, SB-203580 powerfully suppressed double-stranded RNA-mediated production of TNFα, IL-1β and MIP-1α, but not of IFNβ, IL-15, MIP-1β, RANTES or liver and activation-regulated chemokine (LARC), as has been demonstrated in other cell cultures.[123] As described above, p38 MAPK activation is apparently essential for LPS-induced pro-inflammatory cytokine expression. However, SB-203580 has been shown to powerfully

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Table II. Main characteristics of clinically proven small-molecule inhibitors of p38 MAPK activity

Compound	Development phase	Therapeutic targets	Mechanism of action	Originator(s)
Nabidiolex® (cannabidiol/ Δ9-tetrahydrocannabinol)[a]	Launched 2005	Inflammatory bowel disease, multiple sclerosis, rheumatoid arthritis, neuropathic pain, diabetic neuropathy and psychosis	Multitargeted inhibitor, particularly against p38 MAPKs, NOS-2, β-amyloid proteins. Cannabinoid receptor agonist	GW Pharmaceuticals
Clomethiazole (NEX-002)	Launched 1957	Treatment of acute ischaemic stroke, restlessness, agitation and delirium in the elderly, epilepsy	p38 MAPK inhibitor and GABA(A) receptor modulator	AstraZeneca
Succinobucol (AGI-1067, AGZ-1067)	Phase III	Atherosclerosis, restenosis, diabetes mellitus	MAPK-targeted inhibitor, especially against p38 MAPK (VCAM-1, CCL2 expression inhibitor), SAPK-1/2 and ERK-1/2	AtheroGenics
TAK-715	Phase II/III	Rheumatoid arthritis	p38 MAPK-targeted inhibitor. TNFα production inhibitor	Takeda Pharmaceuticals
Pamapimod (RO-440-2257)	Phase II	Rheumatoid arthritis	p38 MAPK-targeted inhibitor. IL-6 and TNFα production inhibitor	Hoffmann-LaRoche
SB-203580	Phase II	Arthritis and inflammatory bowel disease	SAP/Jun and p38 MAPK inhibitor. Calcium channel activator	GlaxoSmithKline
VX-745	Phase II	Myelodysplastic syndrome and rheumatoid arthritis	p38 MAPK-targeted inhibitor	Vertex
Doramapimod (BIBR-796)	Phase II	Psoriasis, inflammatory bowel disease, rheumatoid arthritis	MAPK-targeted inhibitor, especially against p38 MAPK and SAPK2	Boehringer Ingelheim
VX-702 (KVK-702)	Phase II	Inflammatory bowel disease, rheumatoid arthritis, disorders of the coronary arteries and atherosclerosis, osteoarthritis	p38 MAPK-targeted inhibitor	Vertex
Talmapimod (SCIO-469)	Phase II	Myelodysplastic syndrome therapy, rheumatoid arthritis, multiple myeloma	p38α MAPK-targeted inhibitor	Scios
RPR-200765A	Phase II	Rheumatoid arthritis	p38 MAPK-targeted inhibitor	Aventis
RO-3201195	Phase I	Rheumatoid arthritis	p38 MAPK-targeted inhibitor. IL-1β/6 and TNFα production inhibitor	Hoffmann-LaRoche and Roche
SB-242235	Phase I	Arthritis	p38 MAPK-targeted inhibitor	GlaxoSmithKline
RWJ-67657	Phase I	Arthritis and inflammatory bowel disease	p38 MAPK-targeted inhibitor. IL-1β and TNFα production inhibitor	R.W. Johnson

Continued next page

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Table II. Contd

Compound	Development phase	Therapeutic targets	Mechanism of action	Originator(s)
HEP-689 (SB-235699)	Phase I	Nonspecific inflammation, psoriasis, atopic dermatitis	p38 MAPK-targeted inhibitor	GlaxoSmithKline
SB-281832	Phase I	Inflammatory bowel disease, rheumatoid arthritis, allergy, asthma	p38 MAPK-targeted inhibitor	GlaxoSmithKline
R-1487	Phase I	Rheumatoid arthritis	p38 MAPK-targeted inhibitor	Roche
AMG-548	Phase I	Arthritis	p38 MAPK-targeted inhibitor. TNFα production inhibitor	Amgen
EO-1606	Phase I	Atopic dermatitis	p38 MAPK-targeted inhibitor	Leo
SD-06	Phase I	Arthritis	p38 MAPK-targeted inhibitor	Pfizer

GABA = gamma-aminobutyric acid; **SAP** = stress-activated protein. For all other abbreviations, please refer to text.

suppress IL-1β and IL-6 release, as well as activation of cytosolic PLA2 (cPLA2) and related COX-2 activity in LPS-treated differentiated U937 cells via inhibition of the p38 MAPK signalling pathway.[124]

In some cancer cells, p38 MAPK has been regarded as a potential phosphate donor for the p65 subunit of NF-κB, which, in turn, can be directly activated by stimulation of endothelin receptors. It has been re-

Table III. Small-molecule inhibitors of p38 MAPK activity for which the structures are not yet disclosed

Compound	Development phase	Therapeutic targets	Mechanism of action	Originator(s)
GSK-681323 (SB-681323, GW-681323)	Phase II	Atherosclerosis, rheumatoid arthritis, disorders of the coronary arteries and atherosclerosis, neuropathic pain, COPD	p38 MAPK-targeted inhibitor	GlaxoSmithKline
GW-856553 (SB-856553)	Phase II	Depression, atherosclerosis, lipoprotein disorders, rheumatoid arthritis, COPD	p38 MAPK-targeted inhibitor	GlaxoSmithKline
KC-706	Phase II	Cardiovascular diseases (not specified), psoriasis, rheumatoid arthritis, lipoprotein and dermatological disorders	p38 MAPK-targeted inhibitor	Kemia
ARRY-797	Phase II	Rheumatoid arthritis	p38 MAPK-targeted inhibitor	Array BioPharma
PH-797804	Phase II	Rheumatoid arthritis, neuropathic pain, COPD	p38 MAPK-targeted inhibitor	Pfizer
BMS-582949	Phase II	Psoriasis, atherosclerosis, rheumatoid arthritis	p38 MAPK-targeted inhibitor	Bristol-Myers Squibb
SC-80036	Phase I/II	Arthritis	p38 MAPK-targeted inhibitor	Pfizer
AVE-9940	Phase I/II	Rheumatoid arthritis	p38 MAPK-targeted inhibitor	sanofi-aventis
SCIO-323	Phase I	Arthritis	p38 MAPK-targeted inhibitor	Scios
LEO-15520	Phase I	Dermatological diseases	p38 MAPK-targeted inhibitor	Leo
ARRY-614	Phase I	Haematological cancer, arthritis	p38 MAPK inhibitor and Tie2 receptor antagonist	Array BioPharma
TA-5493	Phase I	Psoriasis, rheumatoid arthritis	p38 MAPK-targeted inhibitor	Mitsubishi Tanabe Pharma
AKP-001	Phase I	Inflammatory bowel disease	p38 MAPK-targeted inhibitor	Aska Pharmaceutical

COPD = chronic obstructive pulmonary disease; **Tie2** = endothelium-specific tyrosine kinase-2. For all other abbreviations, please refer to text.

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ported that p38α/β MAPKs and p65/NF-κB jointly form a transcription complex that leads to expression of a number of pro-inflammatory genes, and this expression can be effectively suppressed by SB-203580.[125]

Another study has shown that LPS-stimulated translocation of NF-κB into the nucleus can be totally blocked by SB-203580 via inhibition of p38-signalling cascades.[126] In addition, SB-203580 reduced the level of ICAM-1 and VCAM-1 expression in LPS-induced inflammation of pulp cells.[127] Ongoing speculation about the possible role of p38 MAPKs in the regulation of NF-κB activity was recently strengthened by the finding that SB-203580 can completely inhibit the p38 signalling pathway, significantly reducing NF-κB activation and repressing COX-2 synthesis.[128] It has also been recently shown that p38 MAPK activation results in induction of haem oxygenase (HO)-1, which exerts potent anti-inflammatory effects.[129] A single administration of SB-203580 led to a significant decrease in p38 MAPK/HO-1 activity and related gene expression, including TNFα, IL-6, ICAM-1, cytokine-induced neutrophil chemoattractant (CINC)-1 and MIP-2. In addition, it was recently shown that in human lung epithelial cells (A549) infected with *Mycobacterium bovis* Bacillus Calmette-Guérin (BCG), IL-10 production mediated by p38 MAPK signalling pathways, which in turn are selectively inhibited by SB-203580 in a dose-dependent manner, is not regulated by the (PI3K)/Akt precursor system.[130] It was also demonstrated that, in rats, apoptotic neuronal death caused by systemic administration of glutamate can be completely prevented by SB-203580.[131] The observed effect was directly associated with inhibition of the p38-related signalling cascades leading to TNFα, IL-1β and IL-6 expression, thereby suggesting a potential neuroprotective role of p38 MAPK blockade as part of a common anti-inflammatory therapeutic strategy. In promonocytic U937 cells, SB-203580 significantly blocked VacA-related IL-8 production by inhibiting p38 MAPK-induced phosphorylation of ATF-2 and cyclic adenosine monophosphate response element-binding (CREB).[132] SB-203580 has also been found to completely block the expression of a series of pro-inflammatory genes initiated by influenza virus infection in airway epithelial cells.[133] LPS from Gram-negative bacteria is one of the microbial-associated molecular patterns that initiate the immune/inflammatory response, leading to the tissue destruction observed in periodontitis. Treatment of murine periodontal ligament cells by SB-203580 led to a permanent weakness in p38 MAPK activity that is suggested to be important in LPS-induced receptor activation of NF-κB ligand (RANKL) expression.[134] Furthermore, SB-203580 completely blocks the LPS-induced TNFα expression observed in the mouse macrophage cell RAW 264.7 in normoxic conditions, but does not have this inhibitory effect in hypoxic conditions which, in addition to enhancing LPS-induced p38 MAPK activation, produce an inflammatory response via a mechanism of action that differs from p38 MAPK inhibition.[135]

It was recently shown that C-reactive protein (CRP) is a sensitive marker and mediator of inflammation, and that IL-6 blocking therapy can normalize serum levels of CRP in chronic inflammatory diseases.[136] In this study, systemic administration of SB-203580 resulted in inhibition of the phosphorylation of c-Fos enhanced by IL-1 and IL-6, and diminished expression of the *CRP* gene. SB-203580 and its structural analogue, SB-202190, jointly block p38 MAPK-related signal transduction mechanisms, and SB-202190 treatment could also activate the JNK signalling route, leading to ATF-2 phosphorylation and increased AP-1 DNA binding.[137] Further experiments have clearly demonstrated that SB-202190- or SB-203580-induced JNK activation is dependent on the MLK-3-MKK4/MKK7-related signal transduction pathway.[138] In particular, both compounds induced the phosphorylation and activation of MLK-3. It has also been shown that SB-203580 can effectively suppress TNFα, IL-1β and IL-6 expression in lipid-associated membrane proteins (LAMPg)-induced human monocytic cells (specifically THP-1), that produce various pro-inflammatory cytokines.[139] The enormous therapeutic potential of SB-203580 is not restricted solely by the scientific exercises listed

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above. There are a number of cell-based and animal studies that strongly suggest that SB-203580 is the most promising drug candidate that can be successfully applied as an alternative therapeutic tool in chronic and/or acute inflammation.

Doramapimod

Doramapimod (BIRB-796) is a promising drug candidate that inhibits all p38 MAPK isoforms *in vitro* and *in vivo,* and is undergoing advanced clinical trials for the treatment of inflammatory diseases.[140] Doramapimod has picomolar affinity for these isoforms and low nanomolar inhibitory activity in cell culture. This compound has recently been reported to block the production of TNFα and IL-1β via the p38 MAPK pathway.[141,142] In humans, doramapimod significantly inhibited LPS-induced p38 MAPK activation in the leukocyte fraction of volunteers at low (50 mg) and moderate (600 mg) doses (oral administration).[143] This inhibition resulted in suppression of several pro-inflammatory genes, including those for TNFα, IL-6, IL-10 and IL-1-receptor (IL-1R). In addition, p38 MAPK inhibition diminished leukocyte responses, including neutrophilia, release of elastase-α_1-antitrypsin complexes, and upregulation of CD11b with downregulation of L-selectin. Finally, blocking p38 MAPK decreased CRP release. In the previous study using the same experimental conditions, doramapimod was found to strongly inhibit the LPS-induced reduction of neutrophil CXCR-1 and -2 expression in volunteers receiving the higher dose of the compound tested.[144]

Several computational studies have been conducted to reveal key ligand-protein interactions observed in doramapimod/p38 MAPK complexes.[145-147] For example, doramapimod, as an allosteric inhibitor, binds at a site used by the purine moiety of ATP and extends into a 'selectivity pocket' that is not used by ATP.[147] Thus, prior to doramapimod binding, the kinase undergoes a reorganization of the activation loop, exposing a critical binding domain. Whereas many p38 MAPK inhibitors bind only in the purine site of the enzyme, remaining in a 'DFG-in' conformation, doramapimod displaces the aspartate168-phenylalanine169-glycine170 motif at the start of the activation loop, thereby promoting a 'DFG-out' conformation.

VX-702

VX-702 is one of a series of second-generation, orally active p38 MAPK small-molecule inhibitors designed for the treatment of uncontrolled inflammation, RA and several cardiovascular diseases.[148] This compound was originally developed by Vertex Pharmaceuticals Inc. (Cambridge, MA, USA) in collaboration with Kissei Pharmaceutical Co. Ltd (Matsumoto City, Japan) and currently this compound is being validated in advanced clinical trials. Systemic inflammation has been shown to be a contributing factor to the instability of atherosclerotic plaques in patients with acute coronary syndromes. *In vitro* studies have shown that VX-702 1 μmol/L completely inhibited activation of p38 MAPK by thrombin, a synthetic hexapeptide sequence of serine, phenylalanine, leucine, leucine, arginine and asparagine (SFLLRN), a protease-activated receptor-4 peptide agonist (AYPGKF), U46619 and collagen in human platelets but had no effect on platelet aggregation.[149] This observation suggests that p38 MAPK blocking does not affect thromboxane production in human platelets.

RPR-200765A

Following the pioneering work conducted at SmithKline Beecham (Philadelphia, PA, USA) and elucidation of the roles of p38 with potent and selective inhibitors such as SB-203580, many pharmaceutical companies have embarked upon p38 synthetic programmes, as indicated by the ever-increasing number of patents in this domain. For example, at Aventis (Bridgewater, NJ, USA), a rapid parallel synthesis project led to identification of RPR-200765A and related structures (RPR132331, RPR200765 and RPR203494) as potent and selective p38 MAPK inhibitors.[150] RPR-200765A strongly inhibits LPS-induced release of TNFα *in vitro* in mononuclear phagocytes and human monocytes (EC_{50} = 110 nmol/L) as well as *in vivo* in rats and Balb/c mice (EC_{50} = 6 mg/kg).[151] At oral doses of between 10 and 30 mg/kg/day, RPR-200765A reduces disease incidence and progression in the rat

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streptococcal cell wall arthritis model when administered in either prophylactic or therapeutic dosing regimens. As a mesylate salt (stable monohydrate), this compound shows enhanced oral bioavailability (F = 50% in rats) and excellent chemical stability. Data from the streptococcal cell wall disease model suggest that RPR-200765A could exhibit a profile of disease-modifying activity in RA patients that is not observed with current drug therapies.

RO-3201195

Development of RO-3201195 commenced when a novel class of highly selective N-phenyl-1H-pyrazole-based inhibitors of p38 MAPK was recently discovered during high throughput screening. Based on x-ray crystallographic data, the presence of a unique hydrogen bond between the exocyclic amine of the inhibitor and threonine 106 of unphosphorylated p38α was further established. The data obtained were used to optimize the potency and physicochemical properties of the series studied. During optimization screening it was found that incorporation of the 2,3-dihydroxypropoxy moiety in the pyrazole core resulted in a compound, RO-3201195, with excellent drug-like properties, including high oral bioavailability. Currently, this compound is being increasingly regarded as an orally bioavailable and highly selective inhibitor of p38.[152]

SB-242235

SB-242235 is a potent and selective p38 MAPK inhibitor with promising therapeutic modalities. This compound can be properly regarded as an effective therapeutic tool against cytokine-mediated diseases such as autoimmune or inflammatory diseases. Previous pharmacokinetic studies have revealed that SB-242235 is metabolically stable in rat, dog, monkey and human hepatic microsomes, isolated hepatocytes and liver slices *in vitro*.[153,154] In rats with adjuvant-induced arthritis, SB-242235 significantly reduced LPS-stimulated serum levels of TNFα with a median effective dose of 3.99 mg/kg.[155] SB-242235 inhibited paw oedema at doses of 30 mg/kg and 10 mg/kg per day by 56% and 33%, respectively. In addition, serum IL-6 levels were significantly decreased in adjuvant-induced arthritis rats treated with the 60 mg/kg dose of the tested compound.

SB-242235 and its structural analogue SB-203580 strongly inhibited cytokine-induced MMP-13 expression in a dose-dependent fashion while having a somewhat weaker inhibitory effect on MMP-1 expression in human chondrosarcoma cell lines (SW1353 and JJ012) and human articular chondrocytes.[156] In contrast, inhibitors of ERK and JNK pathways did not inhibit the expression of either MMP. SB-242235 was also found to significantly reduce arthritis scores under chronic pristane-induced arthritic conditions in mice.[157] The observed effect was directly associated with blockage of TNFα, IL-1β and IL-6 expression via p38 MAPK inhibition.

It has been reported that SB-242235 effectively blocked activation of the p38 MAPK signalling cascade and abolished MAPKAPK-2 kinase activity and phosphorylation of heat shock protein (HSP)-27 in SKH-1 mice, providing significant protection against UVB irradiation.[158] Furthermore, this compound strongly inhibited the expression of several pro-inflammatory cytokines including IL-6, KC and COX-2.

RWJ-67657

Acting directly against p38 MAPK, RWJ-67657 strongly inhibits TNFα accumulation and activity sustained by LPS in human PBMCs with an IC_{50} of 3 nmol/L, as well as TNFα release from PBMCs treated with the superantigen staphylococcal enterotoxin B (a T-cell stimulus), with an IC_{50} value of 13 nmol/L.[159] This compound was approximately 10-fold more potent then SB-203580 in all p38-dependent *in vitro* systems tested. RWJ-67657 inhibited the enzymatic activity of recombinant p38α and p38β, but not p38γ or p38δ, *in vitro* and had no significant activity against a variety of other enzymes. In contrast, SB-203580 significantly inhibited the tyrosine kinases p56 lck and c-Src (IC_{50} = 5 μmol/L). RWJ-67657 was not found to inhibit T-cell production of IL-2 or IFNγ and did not inhibit T-cell proliferation in response to mitogens. After oral administration, RWJ-67657 inhibited TNFα production in LPS-injected mice (87% inhi-

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bition at 50 mg/kg) and in rats (91% inhibition at 25 mg/kg).[160]

RWJ-67657 was evaluated against RA in monocyte-derived macrophages.[161] In this study, a strong inhibition of TNFα was registered at pharmacologically relevant concentrations of the compound. Inhibition of mRNA expression of IL-1β, IL-8 and COX-2 was also observed. Furthermore, it was clearly shown that monocyte-derived macrophages have a high constitutive production of MMP-9, which is not affected by p38 MAPK inhibition. In rheumatoid synovial fibroblasts, RWJ-67657 at 1 μmol/L significantly reduced MMP-1 production while the tissue inhibitor of matrix metalloproteinase (TIMP)-1 level remained stable.[162] Significant inhibition of IL-6/8 and COX-2 protein production was observed at 0.1 μmol/L and 0.01 μmol/L, respectively.

It was suggested that inhibition of the p38MAPK-related signalling cascades by RWJ-67657 might be a promising therapeutic tool to intervene in the deranged immune response in sepsis and other inflammatory diseases. *In vivo*, after single-dose administration of RWJ-67657, key physiological parameters, for example temperature and blood pressure, remained at baseline level in response to endotoxin.[163] Inhibition of TNFα, IL-6 and IL-8 response was dose dependent. It should be especially noted that there was no drug-related toxicity.

A study that evaluated the inhibition potency and kinetics of RWJ-67657 found that the dissociation constant (K_d) measured for RWJ-67657 in the complex with p38 MAPK was 22 nmol/L.[164] The values obtained closely matched the IC_{50} values observed in a cell-based TNFα assay, implying that p38 plays a critical role in TNFα release. Both the pharmacokinetic and pharmacodynamic profiles of RWJ-67657 have also been determined by Parasrampuria and coworkers[165] after a single oral dose (0.25–30 mg/kg) of the drug. This study also demonstrated that RWJ-67657 has acceptable safety and pharmacokinetics that warrant further investigation of its use as a promising anti-inflammatory agent.

The therapeutic potential of RWJ-67657 against uncontrolled inflammation was thoroughly investigated in terms of the drug's effects on *in vivo* LPS-induced monocyte cytokine production and monocyte LPS-hyporesponsiveness by Faas et al.[166] These investigators found that single oral administration of RWJ-67657 at increasing dosages (0–1400 mg) led to a corresponding decrease in levels of IL-1β, TNFα and IL-12 in a dose-dependent manner. Treatment with RWJ-67657 also reversed LPS-hyporesponsiveness.

A single oral dose of RWJ-67657 (350 mg) prevented neutrophil and endothelial activation after endotoxin infusion in healthy volunteers.[167] It has also been reported that RWJ-67657 caused a moderate therapeutic effect on ICAM-1 and VCAM-1 expression in endothelial cells, whereas significant reductions in mRNA expression and protein production of the inflammatory cytokine IL-6 and the chemokines IL-8 and MCP-1 were demonstrated.[168] Thus, treatment with RWJ-67657 could lead to reduced leukocyte infiltration by reducing E-selectin expression and chemokine production. Finally, RWJ-67657 has been shown to be a promising drug candidate for COPD[169] and sepsis therapy.[170]

Talmapimod

Talmapimod (SCIO-469) [table II] is a novel small-molecule p38 MAPK inhibitor currently being evaluated in a phase II clinical trial by Scios Inc. (Raritan, NJ, USA) for the oral treatment of RA.[171] This compound was recently described in comprehensive reviews by Lee and Dominguez[172] and Dominguez et al.[173]

Despite the strong rationale for p38 MAPK inhibitors in inflammatory disease, direct proof of concept in the clinic has yet to be demonstrated. Most compounds have been found to have dose-limiting adverse effects. Indeed, development of p38 inhibitors has been slow, probably because of toxicological problems, which might explain why only a few inhibitors have advanced into clinical development. Nevertheless, the strategic role of MAPKs in inflammation makes them attractive biological targets for new alternative therapies, and

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efforts are continuing to identify novel, more selective inhibitors for inflammatory diseases.

1.3 Janus Protein Tyrosine Kinase (JAK)/Signal Transducers and Activators of Transcription (STAT) Signalling Pathway

The rapidly expanding knowledge underpinning the cytokine transcription factor network has 'shed a bright light' on the acute and chronic inflammatory response. JAKs and STAT are critical components of many cytokine receptor systems that regulate cell growth, survival, proliferation, haematopoiesis and pathogen resistance. It was recently revealed that the JAK/STAT signalling network is observed in three major cell types involved in inflammatory responses, namely T cells, neutrophils and macrophages, and plays a critical role in pro-inflammatory cytokine production. JAK belongs to a family of non-receptor protein tyrosine kinases (PTKs) comprising JAK-1, JAK-2, JAK-3 and non-receptor PTK-2 (TYK-2). STATs are latent cytoplasmic transcription factors that become activated after recruitment to an activated receptor complex. A series of seven separate STAT proteins are present in a wide variety of cell types, including cells of epithelial origin.[174] These include STAT-1 to -6, including STAT-5a and STAT-5b, which are encoded by distinct genes. For example, STAT-1 mediates a pro-inflammatory response to activation of the IFNγ receptor on the cell surface by IFNγ. STAT-3 participates in the signalling pathways for many cytokines in various cells and organs that are regulated by the suppressors of cytokine signalling (SOCS) family, including SOCS3 (see below).[175] In addition, different isoforms of several STATs have been identified to date. The JAK/STAT signalling pathways are regulated by a vast array of intrinsic and environmental stimuli, which can add plasticity to the response of a cell or tissue.[176]

Mechanistically, JAK/STAT signalling is relatively simple, with only a few principal components (figure 3). A variety of ligands including cytokines, hormones and growth factors, as well as their specific receptors such as cytokine receptors, EGFR, GPCR and IFN receptors, activate the JAK/STAT pathway through the multimerization of receptor subunits such as STATIP (STAT-Interacting Protein) and TYK-2. For some ligands, such as erythropoietin and growth hormone, these subunits are bound as homodimers while, for others, such as IFNs and ILs, the receptor subunits are heteromultimers. For signal propagation, the cytoplasmic domains of two receptor subunits must be associated with JAK tyrosine kinases. JAK activation occurs upon ligand-mediated receptor multimerization because two JAKs are brought into close proximity, allowing trans-phosphorylation. The activated JAKs subsequently phosphorylate principal targets, including both the receptors and the major substrates, STATs, leading to the dimerization of STATs through interaction with a conserved Src homology 2 (SH2) domain. As shown in figure 3, different JAKs and STATs are activated by different ligands via corresponding receptors. Phosphorylated STAT dimers then translocate into the nucleus by a mechanism involving importin and the Ran nuclear import pathway. In the nucleus, STAT transcriptional complexes bind to specific regulatory regions on DNA to activate or repress transcription of target pro-inflammatory genes. Thus, the JAK/STAT cascade provides a direct mechanism for translating an extracellular signal into a transcriptional response. In addition, the receptor tyrosine kinase (RTK) family that normally regulates the Ras/Raf/MEK/ERK-related signalling cascades including p38 MAPK pathway can also induce the JAK/STAT signal transduction via cytokine and/or IL receptors.

In addition to JAK/STAT pathway effectors, there are three major classes of negative regulator: SOCS, protein inhibitors of activated STATs (PIAS) and protein tyrosine phosphatases (PTPs), including CD45 and Src homology phosphatase (SHP)-1. SHP-1 is the best characterized member of the PTPs, contains two conserved SH2 domains and can bind to either phosphorylated JAKs or phosphorylated receptors to facilitate dephosphorylation of these activated signalling molecules. SOCS proteins are a family of at least eight members containing an SH2 domain and a SOCS box at the C-terminus. The hallmark of the SOCS family is the SOCS box,

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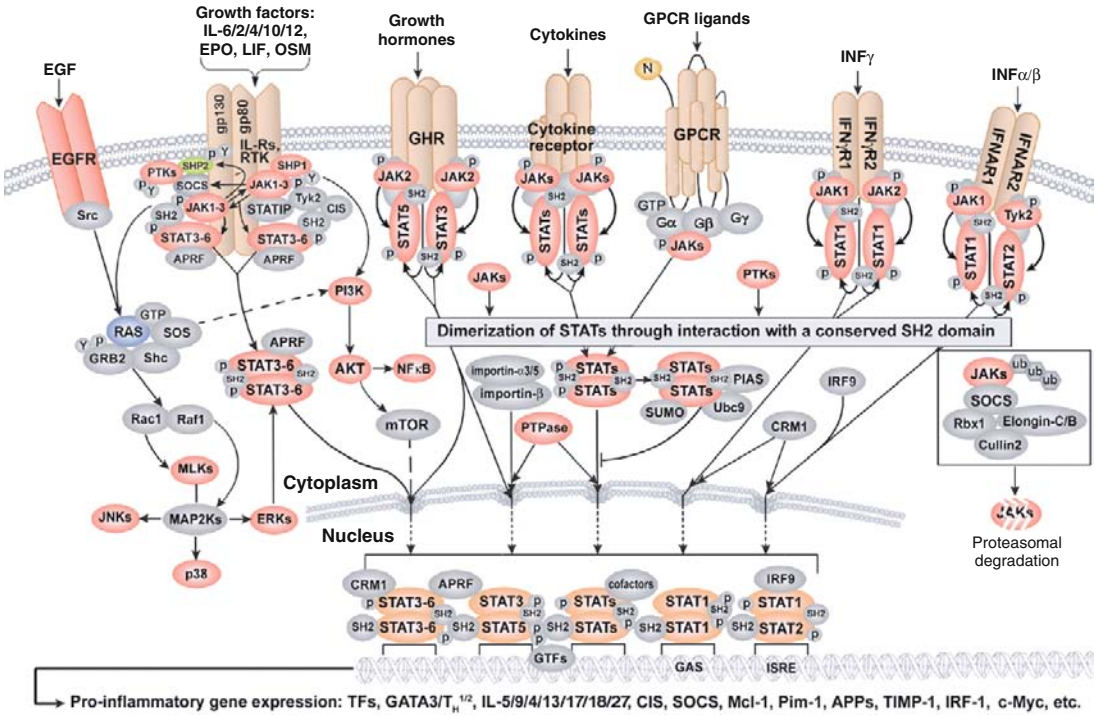

Fig. 3. The Janus protein tyrosine kinases and signal transducers and activators of transcription (JAK/STAT) signalling pathways implicated in inflammation. Please refer to text for definition of relevant abbreviations.

which mediates interaction with the elongin-B/C complex and couples the SOCS and associated target proteins JAKs to the proteasomal protein degradation pathway.

The elongin complex also recruits the really interesting new gene (RING) finger protein ring box (RBX)-1 to form a multiprotein conglomerate that imitates E3 ubiquitin ligase functions. Together with an ATP-dependent ubiquitin-activating enzyme (E1) and a ubiquitin-conjugating enzyme (E2), this complex acts to tag proximal proteins with polyubiquitin chains followed by their subsequent degradation by the proteasome. In addition, JAK/STAT signalling also indirectly promotes Ras signalling through transcriptional activation of SOCS3. Thus, SOCS3 binds Ras GTPase activating protein (ras-GAP), a negative regulator of Ras signalling, and reduces its activity, thereby promoting activation of the Ras pathway. The RTK pathway also promotes JAK/STAT signalling by at least two mechanisms: first, activation of some RTKs, including EGFR and platelet-derived growth factor receptor (PDGFR), results in JAK-independent tyrosine phosphorylation of STATs, mainly by the Src kinase, and second, RTK/Ras pathway stimulation causes downstream activation of MAPK, which in turn specifically phosphorylates a serine near the C-terminus of most STATs, leading to its activation and related transcription.

1.3.1 JAK/STAT Inhibitors

Several small-molecule agents have been shown to regulate JAK/STAT signalling pathway (table IV and table V). Many of these compounds have already been launched on the market as drugs acting against various inflammatory conditions while others are currently being evaluated in different clinical trials.

Leflunomide, for example, is widely used to treat RA and fibrosis. In the HaCaT human keratinocyte cell line, leflunomide was found to significantly

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Table IV. Therapeutically active small-molecule inhibitors of JAK and STAT activities

Compound	Development phase	Therapeutic targets	Mechanism of action	Originator(s)
Leflunomide (HWA-486, SU-101)	Launched 1998	Ovarian, brain and prostate cancers, rheumatoid arthritis, HIV-infection and transplant rejection	Multitargeted inhibitor, especially against JAK-3 and STAT-6	Lepetit and sanofi-aventis
Lestaurtinib (CEP-701, SPM-924)	Phase II/III	Psoriasis, haematopoiesis, prostate and pancreatic cancers, neurological cancer and myeloid leukaemia	Multitargeted inhibitor, especially against JAK-3, RET, TRKA and FLT3 (FLK-2/STK-1)	Kyowa Hakko
CP-690550	Phase II	Psoriasis, inflammatory bowel disease, rheumatoid arthritis, asthma and transplant rejection	JAK-3-targeted inhibitor	Pfizer
Tozasertib (MK-0457, VX-680)	Phase II	Nonspecific and cancer-associated inflammatory diseases	Multitargeted inhibitor, especially against Aurora-C and JAK-2	Vertex
TG-101348	Phase I/II	Cancer and cancer-associated inflammatory diseases	JAK-2 and FLT3 (FLK-2/STK-1) inhibitor	TargeGen
LS-104	Phase I	Cancer-associated inflammatory diseases	JAK-2, BCR-Abl and FLT3 (FLK-2/STK-1) inhibitor	Hospital for Sick Children
Bosutinib (SKI-606)	Phase III	Cancer and cancer-associated inflammatory diseases, ischaemic stroke	Multitargeted inhibitor, especially against STAT-5 and Src, BCR-Abl, Abl kinases	Wyeth Pharmaceuticals
Atiprimod	Phase II	Bone resorption, endocrine and colorectal cancers, multiple myeloma, inflammatory bowel disease, rheumatoid arthritis	Multitargeted inhibitor, particularly against STAT-3 (IL-6 production inhibitor) and PKB/Akt. Apoptosis inducer (caspase-3/9 activator)	AnorMED and GlaxoSmithKline
ABT-869 (AL-39324)	Phase II	Cancer and cancer-associated inflammatory diseases	Multitargeted inhibitor, particularly against STAT-5, ERK, CSF-1-receptor (c-FMS), PDGFRβ, VEGFR-2 (FLK-1/KDR), FLT3 (FLK2/STK1)	Abbott

FLK-2 = fetal liver kinase-2; **RET** = rearranged during transfection (proto-oncogene); **PKB** = protein kinase B; **STK-1** = stem cell tyrosine kinase 1; **TRKA** = tropomyosin-related kinase A. For all other abbreviations, please refer to text.

inhibit IL-4- and IL-13-enhanced production of chemokine (C-C motif) ligand (CCL)-26.[177] The results obtained suggest that keratinocytes are involved in the migration of chemokine (C-C motif) receptor (CCR)-3-positive cells such as eosinophils in a T_h2-dominant situation such as atopic dermatitis. In addition, leflunomide powerfully inhibited the deposition of type I collagen in hepatic stellate cells and the proliferation of primary cells of this type by interrupting the three proliferative signal transduction pathways *in vitro*, including the primary target JAK/STAT and related MAPK and (PI3K)/protein kinase B (Akt) signalling cascades, thereby providing a novel insight into the mechanisms by which leflunomide may act in liver fibrosis.[178]

Lestaurtinib and Tozasertib

Lestaurtinib (CEP-701) and tozasertib (MK-0457) are being studied in a phase II/III clinical trial in patients with treatment-resistant chronic myelogenous leukaemia (CML) or Philadelphia chromosome-positive acute lymphoblastic leukaemia. MK-0457 was originally developed as a small-molecule inhibitor of Aurora kinases A, B and C. However, recent screening data showed that MK-0457 inhibits JAK-2 with an IC_{50} of 123 nmol/L for wild-type JAK-2 and 295 nmol/L for the JAK-2 V617F activating mutation.[179] Lestaurtinib also inhibited wild-type JAK-2 kinase activity (JAK-2/STAT-5 signalling pathway) in the same kinase assay with an IC_{50} of 1 nmol/L *in vitro*.[180] Although these compounds are commonly targeted

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Table V. Small-molecule inhibitors of JAK and STAT for which structures are not yet disclosed

Compound	Development phase	Therapeutic targets	Mechanism of action	Originator(s)
INCB-18424 (INCB-018424)	Phase II	Psoriasis, thrombocytopenic anaemia, haematological and prostate cancers, multiple myeloma, rheumatoid arthritis, PMF and post-PV/ET MF	JAK-2-targeted inhibitor	Incyte
AT-9283	Phase I/II	Cancer-associated inflammatory diseases	Multitargeted inhibitor, especially against JAK-2, Aurora-A/B, BCR-Abl kinases	Astex
R-348	Phase I	Psoriasis, autoimmune diseases, multiple sclerosis, rheumatoid arthritis and transplant rejection	JAK-2-targeted inhibitor	Rigel
XL-019	Phase I	Nonspecific inflammation, haematopoiesis	JAK-2-targeted inhibitor	Exelixis

PMF = primary myelofibrosis; **PV/ET MF** = post-polycythaemia vera/essential thrombocythaemia. For all other abbreviations, please refer to text.

for the treatment of leukaemia and myeloproliferative disorders, they are also being considered as potential small-molecule drug candidates against different inflammatory diseases.

CP-690550

CP-690550 is a novel, selective small-molecule inhibitor of JAK-3 kinase activity with promising anti-inflammatory potential. JAK-3 has been shown to play a key role in pro-inflammatory cytokine signalling via IL-2/4/7/9/15/21. For example, in a murine model of allergic pulmonary inflammation, this compound potently inhibited IL-4-induced upregulation of CD23 (IC_{50} = 57 nmol/L) and class II major histocompatibility complex (MHCII) expression (IC_{50} = 71 nmol/L) in murine B-cells.[181] Animals treated with CP-690550 have exhibited significant reductions in bronchoalveolar lavage fluid (BAL) eosinophils and levels of IL-13 and eotaxin following ovalbumin aerosol exposure.[182,183] Consequently, CP-690550 represents an intriguing novel therapy for treatment of allergic conditions associated with airway eosinophilia, including asthma and rhinitis. CP-690550 also effectively inhibited a murine mixed lymphocyte reaction with an IC_{50} = 91 nm *in vitro*. [184] Furthermore, the ability of CP-690550 1.5–15 mg/kg/day to extend cardiac allograft survival in mice suggests that this agent may represent a new treatment for prevention of transplant rejection. In addition, CP-690550 has demonstrated impressive immunosuppressive potency in nonhuman primates when used in combination with mycophenolate mofetil, with implications for the treatment and prevention of kidney allograft rejection.[181] The numerous and severe adverse effects (e.g. mutations and perturbations across the entire signalling route) associated with all current immunosuppressive therapies justify a search for drugs with better efficacy and safety profiles. Importantly, in several preclinical models, CP-690550 has demonstrated high efficacy in the prevention of allograft rejection with a narrow adverse-effect profile.[185]

TG-101348

TG-101348, a potent, selective and orally bioavailable inhibitor of JAK-2, is currently in clinical trials against myeloproliferative and inflammatory diseases.[186] TG-101348 was originally identified using rational design techniques and an extensive medicinal chemistry effort designed to optimize potency, selectivity and pharmaceutical and pharmacokinetic properties. When tested against a phylogenetically diverse panel of 223 kinases, it was found that TG-101348 inhibited JAK-2 activity with an IC_{50} of 3 nmol/L, while in primary and cultured cells, this compound inhibited JAK-2 driven cellular activity with an EC_{50} ranging from 50 to 300 nmol/L.[187,188] The effect of TG-101348 on cell proliferation strongly correlated with reduced levels of

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phosphorylated STAT-5, suggesting that the observed pharmacological effect was mediated by inhibition of the JAK/STAT signalling pathway. In addition, TG-101348 showed therapeutic efficacy in a murine model of myeloproliferative disease induced by the JAK-2 V617F[189] and JAK-2 V617F+[190] mutations. Based on these promising efficacy results and favourable pharmaceutical properties and preliminary toxicity findings, TG-101348 is currently being evaluated in advanced clinical trials.

Bosutinib

Bosutinib (SKI-606) was originally developed as a promising antiproliferative agent against CML acting as a dual inhibitor of Src and Abl/BCR kinase activities.[191] It was also found that SKI-606 inhibits phosphorylation of several cellular proteins, particularly STAT-5 (IC_{50} = 10–25 nmol/L). Thus, simultaneous inhibition of the Ras, STAT-5 and PI3K pathways directly coupled to BCR/Abl by V-crk sarcoma virus CT10 oncogene homologue (avian)-like (CrkL) leads to synergistic enhancement of apoptosis in CML cells and significant suppression of pro-inflammatory gene expression. In addition, it was found that in a variety of leukaemic states, BCR/Abl may use a bypass mechanism to activate JAK/STAT signal transduction pathways.[192] Similar activity has been assigned to STI-571, a structural analogue of SKI-606.[193]

Atiprimod

In multiple myeloma cells, atiprimod effectively blocks STAT-3 phosphorylation and colony-forming cell proliferation as well as induces cellular apoptosis by cleavage of caspase-3 and poly (adenosine diphosphate-ribose) polymerase (PARP).[194] During phase I clinical trials, this compound exerted potent anti-inflammatory activities in different animal models and in patients diagnosed with RA. For instance, in multiple myeloma cells, atiprimod powerfully inhibited STAT-3 and -5 activation, thereby blocking expression of IL-6, which in turn contributes to myeloma cell proliferation and survival. In addition, atiprimod has been shown to downregulate the antiapoptotic proteins Bcl-2, Bcl-xL and myeloid cell leukaemia (Mcl)-1. Interestingly, in acute myeloid leukaemia (AML) cells, atiprimod significantly decreased phosphorylation of STAT-3 and -5, and protein levels of JAK-2, whereas gene expression of JAK-2 was not affected.[195]

ABT-869

ABT-869 (a novel multitargeted RTK inhibitor) has been found to effectively block growth and progression of AML cells, particularly expressing a wild-type FMS-like tyrosine kinase (FLT)-3 receptor.[196] FLT3 mutations trigger strong autophosphorylation of the FLT3 kinase domain and constitutively activate several downstream effectors such as the PI3K/Akt, RAS/MEK/MAPK and mammalian target of rapamycin (mTOR)/STAT-5 signalling pathways. Thus, ABT-869 has been found to inhibit phosphorylation of FLT3, STAT-5 and ERK in MV-4-11 and MOLM-13 cells (IC_{50} = 1–10 nmol/L).[197] In addition, ABT-869 significantly downregulates expression of cyclins D/E and Pim-1, but increases expression of p21 and p27 as well as induces apoptosis through downregulation of Bcl-xL and upregulation of Bcl-2-antagonist/killer (BAK), Bcl-2 homology-3 interacting domain death agonist (BID) and Bcl-2-associated death promoter (BAD).[198] It has also been shown that ABT-869 strongly inhibits CSF-1 activity in both enzymatic and cellular assays.[199] For example, in an enzymatic assay, ABT-689 competitively blocked the CSF-1/CSF-1-receptor signalling response with K_i values of 3 nmol/L. The CSF-1/CSF-1-receptor system encoded commonly by the c-Fms proto-oncogene directly regulates macrophage differentiation, survival and function, and thus is an attractive therapeutic target for chronic inflammation and malignant diseases. For example, autocrine regulation by CSF-1 has been reported in macrophages during inflammatory responses and in neoplastic cells.[200]

2. Conclusion

It became abundantly clear that a spectrum of inflammatory disorders include various diseases that are tightly coupled with uncontrolled expression of different pro-inflammatory factors, such as cytokines and chemokines, growth factors and various immune response regulators. Their production and

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activity are, in turn, controlled precisely by different signalling systems, including COX-2, NF-κB, MAPK, JAK/STAT, etc. Because of the significant adverse effects that have now been revealed and elucidated for many COX-2 inhibitors, novel small-molecule regulators of alternative NF-κB, p38 MAPK and JAK/STAT signalling cascades as well as related precursor molecules have received a great deal of attention as promising drug candidates for the treatment of various inflammatory conditions, including RA, psoriasis, multiple sclerosis, COPD and diabetes. Many of these compounds have already been launched in the market while others are currently being evaluated in different clinical trials in the hope of developing novel, effective and, at the same time, safe therapeutics.

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